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THESIS

THE REGULATION OF GASTRIC SECRETION IN THE EDIBLE FROG

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H. SMIT

THE REGULATION OF GASTRIC SECRETION IN THE EDIBLE FROG *(RANA ESCULENTA L.)*

THESIS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKS-UNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS DR. S. DRESDEN, HOOG-LERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE TE VERDEDIGEN OP WOENSDAG 31 OKTOBER 1962 TE 11 UUR

door

HANS SMIT
geboren te Heemstede
in 1926

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Promotor:
PROF. DR. H. P. WOLVEKAMP

Aan de nagedachtenis van mijn vader
Aan mijn moeder

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CURRICULUM VITAE

HANS SMIT, geboren 23 februari 1926 te Heemstede, behaalde in 1946 het einddiploma H.B.S.-B aan het Triniteits Lyceum te Haarlem. In datzelfde jaar liet hij zich inschrijven als student in de biologie aan de Rijksuniversiteit te Leiden. Het candidaatsexamen legde hij af in 1955 en het doctoraalexamen in 1958. Voor dit examen werden onderwerpen bewerkt bij :

PROF.DR D.J. KUENEN : vergelijkend onderzoek naar het voedsel van *Calathus erratus en C.ambiguus* (Coleoptera).

H.SMIT Onderzoek naar het voedsel van *Calathus erratus* Sahlb. en *Calathus ambiguus* Payk. aan de hand van hun maaginhouden. Entomol.Ber.,17 (1957) 199–209.

en een tweede onderwerp : literatuurstudie over de vorming van en de biocoenosen in zuurstof-arme subaquatische milieus.

Onder de titel ''Over de vorming van subaquatische organische sedimenten in zuurstof-arme milieus, en het daarin levende benthos'' werd een rapport samengesteld in opdracht en ten dienste van de Bataafse Petroleum Maatschappij.

PROF.DR W.K.H. KARSTENS : anatomisch onderzoek van de stengel van *Cyperus alternifolius* L.

PROF.DR H.P. WOLVEKAMP : regulatie van pepsine secretie bij *Rana esculenta* L.

In 1957 werd hij aangesteld als assistent bij de Experimentele Zoologie aan het Zoologisch Laboratorium der Rijksuniversiteit te Leiden. Aan ditzelfde instituut volgde in 1959 zijn aanstelling als hoofdassistent en in 1960 als wetenschappelijk ambtenaar.

STELLINGEN

I

In de beschouwingen van THORSON, waarin hij aan edafische factoren de voornaamste rol toekent bij het samenstellen van de biocoenosen die vlakke submariene bodems bewonen, moet het verwaarlozen van de factor zuurstof als een gemis beschouwd worden.

G. THORSON. Verh.dtsch.Zool.Ges. (1952) 276-327.
J.Marine Res., 14 (1955) 387-397.

II

SHEPARD's verklaring, die de acclimatisatie van vissen aan lage zuurstofconcentraties van hun medium toeschrijft aan een toename van de zuurstofcapaciteit van het bloed, is niet overtuigend.

M.P. SHEPARD. J.Fish.Res.Bd.Canada, 12 (1955) 387-446.

III

Als IVY in de titel van zijn publicatie "Proof that histamine is a secretory hormone" suggereert dat histamine een hormoon is voor de secretie van maagsap, dan kent hij aan de proeven van SCHAYER en IVY een te grote bewijskracht toe.

A.C. IVY. (1959) Proc.World Congr. Gastroenterology 1958.
R.W. SCHAYER and A.C. IVY. Amer.J. Physiol., 189 (1957), 193 (1958).

IV

Het bezit van 6 poten bij larven- (en eventueel praelarven-) stasen van sommige Arachniden is een secundair kenmerk; in vergelijking hiermee moet de 8-potige toestand bij nymphen en adulten als primitief worden beschouwd.

V

De familie Coccinellidae vormt een aparte groep van Coleoptera, en zij behoort niet ondergebracht te worden in de familiegroep Clavicornia.

VI

Bij de aanleg en inrichting van horti botanici dient gestreefd te worden naar combinatie van hun wetenschappelijk doel en hun sociale functie in ruimere zin.

VII

De indeling van FAVARGER, die de polyploïde taxa verdeelt in paleo-, meso- en neopolyploïden, betekent weliswaar een verfijning van de cytotaxonomische methode in de historische phytogeografie, maar neemt de bezwaren die aan deze methode verbonden zijn toch niet weg.

C.FAVARGER. Ber.d.Geobot.Inst. Eidg.Techn.Hochsch.Stift.Rübel. 32 (1961) 119-146.

VIII

De door WATTIAUX bij *Drosophila melanogaster* gevonden relatie tussen ontwikkelingsduur en aantal abdominale chaetae is niet een gevolg van het bestaan van een genetische correlatie tussen deze eigenschappen, maar wordt veroorzaakt door overbevolking van zijn cultures.

J.M. WATTIAUX. Nature, 194 (1962) 706-707.

IX

Indien de Nederlandse overheid meent uit sociale overwegingen tot het verstrekken van zogenaamde kinderbijslag over te gaan, dient zij zich niet te onthouden van aanbeveling van het in toepassing brengen van methoden ter regeling van het aantal geboorten.

X

Omdat de hengelsport in Nederland een belangrijke bijdrage levert tot de volksrecreatie, verdient het aanbeveling het verpachten van viswater aan beroepsvissers sterk te beperken.

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CHAPTER I. INTRODUCTION.

1. Presentation of the problem.

There exists an extensive knowledge of gastric secretion and its regulation in mammels. This knowledge has been gained through the investigations of PAVLOV (1949 – 1936) and his associates, and since PAVLOV's time, it has been customary to divide this regulation of secretion into two phases: a reflectory and a chemical phase (humoral phase). The nervus vagus is part of the reflex arc, and cutting the vagus abolishes the secretion which is brought about by conditioned and unconditioned reflexes.

Much work on the humoral phase has been done by IVY and his co-workers. They were able to show that gastric acid secretion is under hormonal control. EDKINS (1906) postulated the presence of a secretory hormone "gastrin", and many attempts to demonstrate the existence of this hormone have been made. There have also been attempts to deny its existence (e.g. RASENKOV, 1928).

In 1919 POPIELSKI discovered the strong stimulating effect of histamine on gastric secretion. Since that time histamine is used as a stimulant for gastric secretion both in physiological investigations and in clinical practice. The controversy as to whether or not histamine is identical with gastrin has been solved mainly by KOMAROV (1938,1942), who was able to prepare a gastrin-containing extract from pyloric mucosa which was practically free from histamine. In the course of years the methods for preparing these gastrin preparations have been considerably improved by a number of investigators (a.o. GREGORY and TRACY, 1961). There is strong evidence that histamine plays a role in the normal physiology of gastric secretion, although the mechanism is not yet clear.

It is known that gastric secretion is under control of the parasympathetic system. Acid secretion, though started by this system, seems to be less closely regulated by it. Pepsin secretion, too, may be partly dependent on humoral influences, though opinions on this point are divided.

BEAUMONT (1833) had already observed that the composition of gastric juice is not the same under different circumstances, and that the elements which constitute gastric juice are separate secretions. PAVLOV and his co-workers, and a number of other investigators, observed that the composition of gastric juice is dependent upon secretory rate, and the kind, strength and duration of the stimulus.

In contrast to the extensive knowledge on gastric secretion in mammals our knowledge of secretory regulation in other vertebrates is limited. There has been some work with Elasmobranchs (DOBREFF, 1927; BABKIN *et al.*, 1935), but the experiments failed to prove the existence of a regulatory mechanism. There is some evidence that secretion in birds is regulated in the same way as in mammals (COLLIP, 1922; FRIEDMAN, 1939; WALTER, 1942).

Nothing seems to be known with certainty about the regulation of gastric secretion in reptiles.

Investigations on gastric secretion in amphibians led to controversial results. KEETON, KOCH and LUCKHARDT (1920) obtained acid secretion following injection of a mammalian gastrin preparation in *Rana catesbyana*, but the extract failed to cause secretion in *Rana esculenta*. (The extract they used probably contained histamine.) SMIRNOFF (1922) is of the opinion that a humoral phase does occur in frogs. POPIELSKI (1929) succeeded in obtaining an increase of gastric juice output after histamine administration in *Rana esculenta* L., but only when the temperature was raised to 37°C. FRIEDMAN (1937) obtained an increase of acid secretion in *R. esculenta* with the aid of histamine.

With regard to nervous influences on secretion in frogs, opinions are divided. SMIRNOFF (1922) and FRIEDMAN (1934) are of the opinion that secretion in *Rana* is regulated via sympathetic nerves. WOLVEKAMP and TINBERGEN (1942), however, thought this doubtful, since cutting the sympathetic chain and severing the connections between sympathetic chain and spinal cord does not inhibit pepsin secretion. Nor was vagotomy followed by inhibition of gastric secretion caused by mechanical stimulation of the stomach wall. CROMBACH, DE JONG and WOLVEKAMP (1958) applied combined vagotomy and sympathectomy in *R. esculenta* L. Because this procedure did not inhibit pepsin secretion, the authors consider the regulation of pepsin secretion a local process, that is, mechanical stimulation of the stomach wall causes secretion either directly, or via a periferal nervous mechanism.

This study aims at ascertaining the role of the periferal nervous mechanism in gastric secretion in *Rana esculenta* L. by means of splanchnectomy and of pharmacological blocking of impulse transmission in the intramural nerve plexus of the stomach. Both pepsin secretion and secretion of acid were investigated. There is a question as to whether nervous regulation of gastric secretion in *Rana* exists

at all. Also, a number of experiments to prove of disprove the existence of a hormonal secretory mechanism in the frog have been performed, and finally, the role which is played by vascular reactions has been investigated.

The similarities and differences between the regulation of gastric secretion in mammals and the frog are discussed.

2. Anatomy of oesophagus and stomach.

a) The oesophagus is a thin walled elastic tube of about 2 cm. length in a medium sized frog weighing about 50 g. The stomach has a thicker wall. It has a length of approximately 4 cm. The pyloric part is narrower than the corpus and shows a rather sharp bend. The stomach is held in place by oesophagus and duodenum only. It is situated on the dorsal side of the liver and to the left of the median. When empty the gastric lumen is very small, but its great elasticity allows the animal to swallow large lumps of food (as for instance a specimen of *Hydrous piceus* L. or other smaller frogs). The inner side of oesophagus and stomach is strongly folded. The longitudinal folds of the oesophagus pass into those of the stomach without interruption . The boundary between oesophagus and stomach is to be seen if these organs are cut lengthwise; the inner side of the oesophagus is whitish, the mucous membrane of the stomach has a brownish-yellow colour.

b) The digestive glands of the frog are situated in oesophagus, corpus and pylorus. The oesophageal glands secrete pepsinogen. SWIECICKI (1876) considered the chief cells of the oesophageal glands to be pepsin secreting cells. LANGLEY (1881) distinguished chief cells and neck cells, which secrete respectively pepsin and mucus. The oesophageal glands contain typical secretory granules (OPPEL, 1896). The differences between oesophageal and gastric glands would not be essential (MACHAN, 1935). LANGLEY demonstrated that the granules in the chief cells of the fundic glands are related to those of the oesophageal glands. BARRINGTON (1942) distinguished two sorts of cells in the corpus glands: chief cells and mucoid neck cells. Zymogenic cells are lacking in the pyloric glands, in which acid secreting parietal cells only are found. In a more recent histological study NORRIS (1959) distinguished four zones in the foregut of *Rana pipiens*: a) oesophagus, b) forestomach (e.g. a transitional zone), c) the fundic portion of the stomach, and d) the pyloric portion. The glands of the oesophagus contain two

types of cells: zymogenic and mucous cells; the latter are mainly present in the neck of the gland. The part which NORRIS calls the forestomach is a zone of approximately 5 mm. just caudal of the oesophago-gastric junction. In this zone the compound tubulo-acinous glands of the oesophagus change into the simple or branched tubulous glands of the fundus. In this area, too, zymogenic cells occur. In the fundic glands two types of cells are to be found: mucous neck cells and fine granulated eosinophilic cells, which resemble the parietal cells in mammals. In the pyloric portion, the glands are shorter and they contain mucous and parietal cells. NORRIS modified BOWIE's method for staining the secretory granules (BOWIE, 1935) because by the method of BOWIE the granulae of the eosinophilic tissue are also stained. This modification results in a more specific staining: only secretory granules of oesophagus and forestomach are stained, the granules in the cells of the fundus remain unstained. Thus the zymogenic cell is only found in oesophagus and forestomach, and parietal cells occur only in fundic and pyloric regions. The glands of all four zones possess mucoid neck cells.

SWIECICKI (1876) stretched the stomach and oesophagus of *Rana*, after which he punched small round pieces from several areas. Of these pieces he measured the peptic activity with the aid of carmin fibrin. In this way it was shown that the greatest amount of pepsin occurs in the oesophagus, whereas in the pyloric part only a very small quantity could be detected.

EXPERIMENT.

To demonstrate that HCl is secreted only in the lower part of the stomach, I performed a simple experiment. An oblong piece of sponge was moistened with water having a pH-value of about 5.5 and some drops of a bromcresol blue solution were then added. This indicator changes its colour from yellow to blue between pH 3.8 – 5.4. The blue sponge was put into the stomach with the aid of a pair of forceps. After approximately 15 minutes it was removed by means of a piece of string which was tied to the sponge and which hung out the frog's mouth. It was found that the lower tip of this oblong sponge had turned yellow while the remaining part kept its blue colour.

It was less easy to obtain conclusive results pertaining to pepsin secretion by this method. A long and thin piece of sponge which had been left in the stomach and oesophagus for one hour was cut transversely into two equal parts after removal from the stomach. Of each part the peptic activity was measured by means of a method

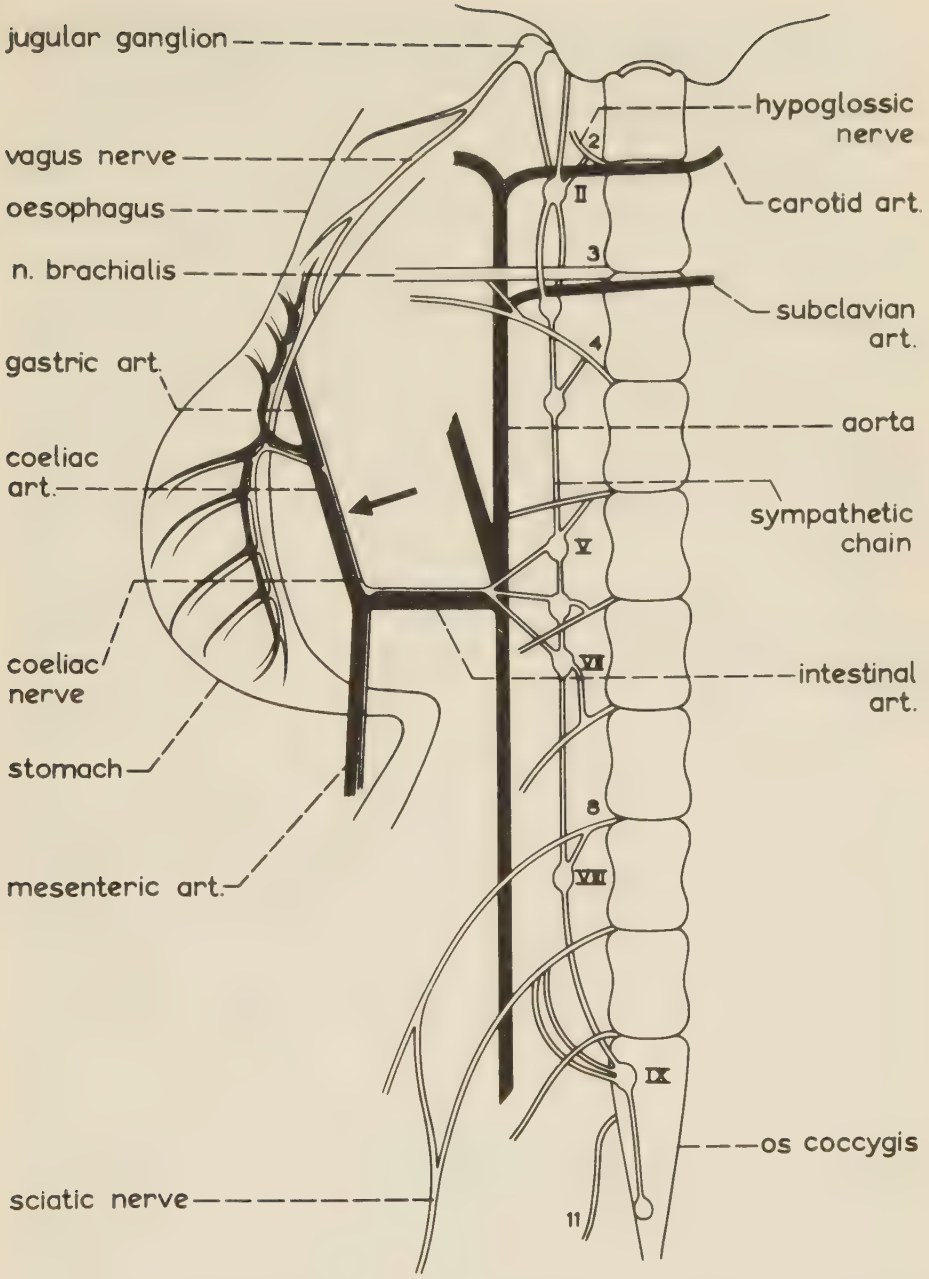

Fig.1. Schematic representation of the stomachal innervation in *Rana esculenta* L. Ventral view. Aorta and stomach are shifted to the right.

which will be described later. The foremost part appeared to contain the greatest amount of pepsin. Since the absorbed gastric juice tended to spread over the entire sponge, this experiment gave no answer to the question of whether or not the stomach itself secretes pepsin.

c) The vessels which supply blood to the oesophagus are the two rami oesophagei of the laryngeal artery, and the oesophageal artery which rises directly from the aorta (ECKER–GAUPP, III,1904). Finally, the unpaired art. gastrica dorsalis, which is a branch of the coeliac artery, approaches from the caudal direction. Arterioles and venules are to be seen in the connective tissue which is situated between the glands and the muscular layer. The stomach is supplied with blood from branches of the dorsal and ventral gastric arteries, which are derived from the coeliac artery, and from branches of the anterior mesenteric artery. Coeliac and mesenteric arteries are the two branches into which the art. intestinalis communis ramifies (see Fig. 1).

d) Oesophagus and stomach possess a double innervation. At the place where the intestinal vagal trunk lies on the musculus petrohyoideus posterior III, it sends two branches to oesophagus and stomach. The sympathetic fibres to the stomach run alongside the two gastric arteries.

> The sympathetic chain is well developed in *Rana*. GAUPP gives the foremost sympathetic ganglion the number "II", because it is connected via rami communicantes with the second spinal nerve. This second nerve is the hypoglossic, which arises from the boundary of medulla oblongata and spinal cord, and which passes between the first and second vertebrae (see Fig.1). In fact, the hypoglossic nerve is the first spinal nerve, but on comparative anatomical grounds it became number II. This point of view is supported by embryological data which show that the first spinal nerve is preformed but degenerates. LANGLEY (1911) ignores the comparative anatomical argument, and considers the hypoglossic nerve the first spinal nerve. Accordingly, his numbering differs from that used by GAUPP. I shall use the terminology of the latter.

e) The wall of oesophagus and stomach contains nerve plexuses, which are considered to cause the automatic contractions, though these contractions are partly regulated by extrinsic nerves (PATTERSON, 1920). OPPEL (1896) used terms analogous to those for mammals, calling the submucosal plexus Meissner's plexus, and the meshwork that lies between the two muscular layers Auerbach's plexus. MARGARETH GUNN (1951) also speaks of a plexus of Meissner, although she has not found any nerve cells in the submucosa. The cells of

the myenteric plexus are usually grouped in ganglia. Only a few vagal fibres extend beyond the stomach approximately 1 cm. over the most oral part of the duodenum.

The capillaries which are located in the subglandular connective tissue are supplied with plexuses (MÜLLER,1908). From this meshwork separate fibres extend to the glands which are surrounded from below by nerve plexuses. GUNN distinguished between motor and sensory cells.

It is clear that an anatomical substrate for presumed local reflexes is present.

3. Stomachal motility as effected by the autonomic nerves.

A number of authors have dealt with the regulating influences of the extrinsic nerve supply on the motility of oesophagus and stomach in the frog. BOTTAZZI (1899) was able to obtain an increase of oesophageal tone by stimulating the vagus. DIXON (1902) distinguished two phenomena : a) tone, and b) spontaneous contractions. Stimulation of the vagus augments the contractions, but, according to DIXON, decreases the tone. HOPF (1911) also found that vagal stimulation augments the motility of the stomach. ITAGAKI (1930) always obtained an increase of the stomachal tone, contrary to the finding of DIXON. On the other hand, the amplitude of the contractions did not change in his experiments. AIKAWA (1931) observed changes in motility during vagal stimulation; in most cases both spontaneous contractions and tone increased, but sometimes there was an inhibitory effect. FRIEDMAN (1935) pointed out that the effect of vagal stimulation depends on the existing tone at the beginning of the stimulation. BOTTAZZI (1899) and CARLSON and LUCKHARDT (1921) were able to increase the peristalsis of oesophagus and stomach by stimulating the sympathetic nerves. YÜH (1931) found sometimes a rise and sometimes a lowering of the tone by sympathetic stimulation. Vagotomy reduces the tone (PATTERSON, 1920; ITAGAKI, 1930). According to PATTERSON, splanchnectomy led to an increase of tone; ITAGAKI, on the other hand, was not able to confirm this result. Total nervous isolation of the stomach, however, did not influence the automatic contractions (PATTERSON, 1920; CARLSON and LUCKHARDT, 1921). Several investigators made use of strips cut from the stomach, from which they made records after addition of drugs. In this way SCHULTZ (1897), YÜH (1931) and EPSTEIN (1932) obtained a lowering of the tone and an inhibition of the automatic movements after treatment of the strips

with atropine. Adrenaline yielded varying results. GRUBER (1923) assumed that the splanchnic nerve sends both excitatory and inhibitory fibres to the stomach. These two sorts of fibres could have a different sensitivity to adrenaline. The fibres which are stimulated by adrenaline in a low concentration might then cause an increase in tone and contraction; the other fibres, which are stimulated by high concentrations of adrenaline, would cause a decrease in tone. AIKAWA (1931) worked with isolated stomachs, which were suspended in a perfusion glass. Ergotamine applied to the outer muscular layer failed to abolish the motor effect of sympathetic stimuli; however, if ergotamine was applied to the muscularis mucosae it caused an inhibition of motility. Adrenaline always inhibited the activity of the muscular layer, but it exerted a motor effect on the muscularis mucosae. After adding ergotamine the mucosa was also inhibited by adrenaline. From this AIKAWA concluded that the sympathetic nerve supply to the outer muscular layers contains only inhibitory fibres, whilst the muscularis mucosae is innervated both by excitatory and inhibitory fibres. FRIEDMAN (1935) used longitudinal and circular strips from the stomach of *Necturus*. He found that adrenaline decreased the tone and inhibited the contractions of longitudinal strips. The circular strips responded to adrenaline with increasing tone and a rise in frequency of contractions. At high concentrations a decrease in tone and frequency took place.

The results of these investigations concerning the control of motility of stomach and oesophagus can be summerized as follows.

1) Vagal stimulation was followed by a) an increase in tone (BOTTAZZI, 1899; CARLSON and LUCKHARDT, 1921; ITAGAKI, 1930; AIKAWA, 1931), b) an increased frequency of contractions (DIXON, 1902; HOPF, 1910; CARLSON and LUCKHARDT, 1921), c) no change of frequency (ITAGAKI, 1930), d) often an increase, but sometimes a decrease in the degree of contraction (AIKAWA, 1931; FRIEDMAN 1935).

2) Sympathetic stimulation, according to some authors, causes an increase in frequency of contractions (BOTTAZZI, 1899; CARLSON and LUCKHARDT, 1921; YÜH, 1931). According to others, an increase in frequency might often occur, but sometimes there is a decrease (YÜH, 1931; AIKAWA, 1931).

3) Vagotomy causes a decrease in tone (PATTERSON, 1920; ITAGAKI, 1930).

4) Sympathectomy causes an increase in tone according to PATTERSON (1920), but no change in tone according to ITAGAKI (1930).

5) Administration of atropine a) diminishes the muscular tone

(SCHULTZ, 1897; YÜH, 1931; EPSTEIN, 1932), b) inhibits the automatic contractions according to SCHULTZ, YÜH and EPSTEIN, c) but according to BECK (1907) it stimulates the spontaneous movements.

6) Administration of adrenaline a) inhibits the movements of the stomach (BECK, 1907; CARLSON and LUCKHARDT, 1921; YÜH, 1931; FRIEDMAN, 1935), b) decreases the tone (HOPF, 1910; YÜH, 1931; EPSTEIN, 1932; FRIEDMAN, 1935), c) but, according to GRUBER (1923), at high concentrations only, while at low concentrations the tone of circular muscles increases. Adrenaline always decreases the tone and inhibits the contractions of the longitudinal muscles (FRIEDMAN, 1935).

The results of these investigations show that the vagus seems to have a stimulatory effect on stomachal motility, whilst the activity of the sympathetic nerves does not always have an inhibitory effect. The antagonism between the two divisions of the autonomic nervous system controlling oesophageal and stomachal motility does not appear to be marked. The tone of the plain musculature is chiefly governed by the intrinsic nerve plexus. The regulatory influence of the extrinsic innervation is superimposed upon the activity of this plexus.

CHAPTER II. MATERIAL AND METHODS.

4. Care and maintenance of the animals.

The frogs were kept in small aquaria with a length of about 20 cm., a width of 14 cm. and a height of 13 cm. Each animal had its own aquarium provided with a label and covered with a glass plate held in place by a piece of brick. These aquaria contained tapwater to a depth of approximately 2.5 cm. Every other day this water was replaced by fresh tapwater, which was previously brought to room temperature. The aquaria were placed in a constant temperature room (20°C.), which was lit during the day. In this way "summer" frogs were procurable troughout the year.

The animals were fed once a week with minced raw ox's or hog's liver mixed with wheat germ. A mouthfull of this mixture was fed at one meal. The meal was served by two persons: one of them opened the frog's mouth, the other fed the animal with the aid of a spatula. Animals may be kept in good health for years in this manner.

5. Stimulation of gastric secretion; collecting the secreted juice.

To obtain the gastric juice, a technique devised by SPALLANZANI (1785), whereby a piece of sponge is pushed into the stomach, has been used. In *Rana*, gastric juice secretion can be produced by mechanical stimulation of the stomach wall caused by the gastric contents (SMIRNOFF, 1922). Entirely inert substances, such as glass beads and pieces of rubber and sponge, are capable of producing secretion (cf. FRIEDMAN, 1934). Accordingly, a piece of sponge placed in the stomach has a double function: it provides a mechanical stimulus, and it absorbs the secreted juice. The sponge used have an oblong shape adapted to the form of the frog's stomach. The sponge is fastened to a piece of string, the other end of which is tied round the frog's foreleg. The sponge is inserted into the stomach through the mouth, and after the appropriate length of time, it can be recovered with the aid of the string.

If the sponge material is homogeneous, one can vary the strength of the stimulus by using sponges of different sizes. Dry weight was used as a measure of size, and therefore no use was made of the irregularly structured natural sponge, but a homogeneous substitute with small pores was employed. In order to denote the strengths

of the stimuli as a function of the sponge's size, the term relative sponge size has been introduced (further indicated as r.s.s.). A sponge of r.s.s. = 1 weighs as many milligrams as the number of grams of the frog: a frog weighing 50 grams "fed" with a sponge of r.s.s. = 2 thus receives a sponge of 100 mgs. However, there is no a priori reason why increases in stomachal capacity and in bodyweight should be exactly proportional, and therefore this method of measuring the mechanical stimulus might lead to erroneous conclusions. The figures in Table 1 confirm this apprehension.

Table 1. Influence of relative sponge size (r.s.s.) on pepsin output in frogs with different bodyweights. (secretion time: 4 hours.)

Bodyweight in grams	Pepsin units	
	r.s.s. = 0.7	r.s.s. = 2.5
30 — 50	17.0 ± 9.4	23.9 ± 7.8
60 — 80	11.6 ± 6.0	41.2 ± 8.0

It appears that the heavier frogs react more strongly to a large sponge than the smaller ones. Thus, either the capacity of the stomach does not increase in proportion to the animal's bodyweight, or the elasticity or sensitivity of the stomach has changed as the animal grew larger. This difficulty in accurately quantifying the mechanical stimulus was remedied by keeping the means and variations in bodyweights of groups of animals between which comparisons were to be made, constant. It is, of course, better still to work with animals of the same weight.

One must take into account the possibility that more gastric juice is secreted than can be absorbed by the sponge. As might be expected, the length of time the sponge remains in the stomach has an effect on pepsin output. This is evident from Figs. 6 and 12, from which it appears that the quantity of juice collected increases with time. The increase in yield progressively diminishes till a final value is reached. From this it might be concluded that after a time secretion stops. However, it is possible that the sponge, becoming saturated, is unable to absorb more gastric juice, although the glands are still secreting. To prevent complete saturation, experiments must be stopped before maximum absorption occurs. Most experiments

has a duration of two hours; in some experiments a period of four hours was chosen. Of course, when choosing a suitable time period, secretory rate had also to be considered.

6. Analysis of gastric juice.

After the required time, the sponge is removed from the stomach and immersed in 5 ml. of HCl 0.01 N. Next, the sponge is squeezed out by means of a glass rod. The peptic activity of this juice is measured by a nephelometric method, devised by BUCHS (1947). As a substrate, edestine is used. This is a vegetable globuline which occurs in seeds of hemp and flax. A 5 per mille solution in 0.02 N HCl serves as a standard; the pH value of this solution is 2.2. At the end of the experiment the non digested protein is precipitated with the aid of a salt. The advantage of BUCH's method lies in the use of an Arabic gum solution, which functions as a protecting colloid and prevents salting out of the protein. If a mixture of gastric juice and substrate is added to a solution of Arabic gum, and then some sulfosalicylic acid is added, the protein forms a homogeneous milky suspension, which is stable for a long time. The degree of turbidity depending on the quantity of undigested protein is measured nephelometrically.

The method is as follows: the sample of gastric juice is mixed with 5 ml. of HCl of pH 2. A test tube containing 5 ml. of an edestine solution of pH 2.2 is heated in a waterbath to 40°C. Next, 1 ml. of the mixture is added to the substrate. After exactly 10 minutes 1 ml. of this substrate-enzyme mixture is pipetted into 10 ml. of an Arabic gum solution (5 per mille in distilled water). About 2.5 seconds later 1 ml. of a 20 per cent sulfosalicylic acid solution is added. The turbidity is measured with a Gallenkamp electric colorimeter. The turbidity caused by a substrate solution to which 1 ml. of 0.01 N HCl is added, instead of the diluted gastric juice, gives the reading corresponding to 100 per cent undigested protein. The reading for distilled water gives the zero value. From the data obtained in this way, the percentage digested protein is calculated. If a colour filter is used which transmits light with a wavelength of about 450 mμ, a linear relationship is obtained between the concentration of the protein solution and the reading of the micro-Ampère meter, so that the percentage of digestion can be calculated directly from the readings.

There is not a linear relationship between the percentage of digestion and the enzyme concentration. Of a 5 ml. sample of gastric juice in HCl, obtained by the method previously described, a series

of dilutions was prepared in such a way that the most diluted portion contained only one part of the original sample in 64 parts. Arbitrarily, the number of pepsin units in the undiluted sample is chosen as 64. The percentage of digestion and the degree of solution are now plotted in a graph (Fig. 2). The curve A appears to be an exponential one; when the enzyme concentration is plotted against the digestion percentage in a logarithmic grid, a straight line is obtained (B). For this line the following formula holds:

$$\log P.U. = 0.0277\ d - 0.3600$$

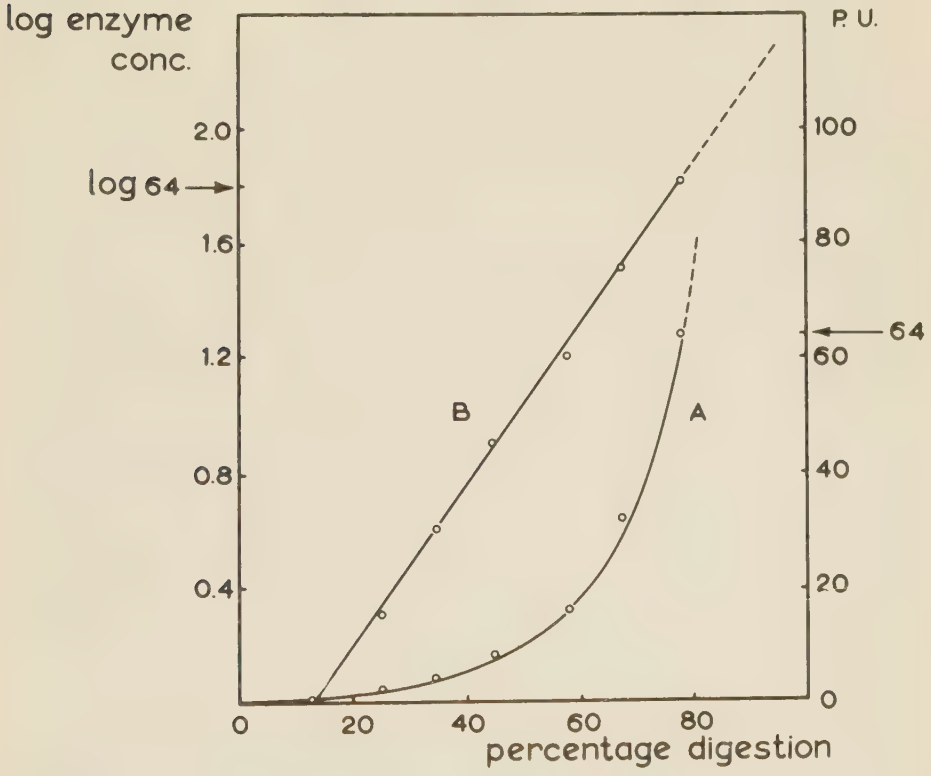

Fig.2. Relation between pepsin concentration and proteolytic activity. Curve A represents the measurements in a linear plot. B shows the same figures plotted in a logarithmic grid.

It is assumed that a definite percentage of digestion found under standardized conditions will correspond with the same pepsin units in every experiment done with gastric juice obtained from different animals. Thus, the peptic activity of a gastric juice sample can be calculated from the digestion percentage (d) by means of this formula.

By establishing the above mentioned logarithmic relationship, the type of reaction is not determined. For kinetic measurements digestion should never have been allowed to proceed as far as it did in a great number of my experiments. In the graph (Fig. 2), accordingly, no linear relationship between the rate of breakdown and enzyme concentration occurs. It is at very low concentrations or extremely short reaction times only that a linear relationship could be expected. This is, however, no drawback, as my sole purpose was to obtain a practically usefull method by which the relative quantity of enzyme in any given sample could be measured.

In those cases where not only the peptic activity of the gastric juice is measured, but the quantity of HCl as well, greater dilutions are used. The sponge containing the gastric juice is first squeezed out in 3 ml. of acidified distilled water of pH 4.7. One ml. of this solution is pipetted into 2 ml. of HCl 0.01 N. Of the latter solution 1 ml. is added to the standard protein solution in order to measure the enzymatic activity. The figures obtained must be multiplied by 2 in order to obtain comparable values.

It is important that the stomach should be entirely empty before the sponge is inserted, because any food present will interfere with the measurement of peptic activity. Without this precaution, a diluted gastric juice sample may exhibit a stronger proteolytic activity than the undiluted sample. The reason for this is probably that, in the diluted sample inhibiting digestion products of food are also diluted. (This is the reason why in METT's method, modified by NIRENSTEIN and SCHIFF (1902), an at least 16-fold diluted digestory mixture is used.) In the method that I used, the gastric juice is approximately 60 to 70-fold diluted. Besides, only frogs which had been starved for at least five days were used. Thus, the chance that food remains would influence the pepsin measurement was reduced to a minimum.

In all series of experiments the averages have a rather large spread. This is without doubt partly due to inaccuracies in the method of standardizing the mechanical stimulation. The stimulus is calculated with relation to the frog's bodyweight, but the size of the stomach would provide a better measure, as the mechanical stimulus depends directly upon the distension of the stomach. In Section 5 this question has already been discussed.

The nephelometric measurements cause another error of approximately 1 per cent. This was shown by the results of 50 randomly chosen double measurements. Since the digestion percentage

is proportional to the log of the enzyme concentration, the error increases exponentially with increasing digestion values (see Table 2).

Table 2. Maximal error in pepsin units (P.U.) at colorimetric measurements of the digestion percentages (d %).

d %	P.U.
10	0.83 ± 0.05
20	1.6 ± 0.1
30	3.0 ± 0.2
40	5.6 ± 0.3
50	10.6 ± 0.7
60	20.0 ± 1.3
70	37.9 ± 2.4
80	71.8 ± 4.6

By duplicating each measurement the reading error wil be somewhat reduced.

Apart from these errors, the magnitude of which can be estimated, differences of secretory response exist in the animals themselves. Individuals differ between one another in responsiveness and also several responses from one individual possess variability. An exsample of this is given in Table 3.

Table 3. Differences of pepsin output between and within individuals. Secretion period 4 hours.

frog No.	weight in grams	P.U. (r.s.s. = 0.5)		P.U. (r.s.s. = 2.5)	
9	81	9.9	13.9	39.4	63.2
36	32	6.2	13.2	24.3	24.3
59	41	6.1	6.8	13.0	13.2
60	72	7.7	20.7	30.4	36.8
67	33	9.9	8.2	12.2	16.0
70	36	10.8	12.0	18.8	18.6
71	24	4.5	8.5	16.6	13.5
72	112	12.8	15.0	22.2	---
mean	54	10.3 ± 2.0		24.2 ± 6.8	

In comparison with the wide variation between individuals, the measuring error is small, and errors caused by inaccuracies in the degree of mechanical stimulation are offset by repetition of experiments with the same specimens. Differences between individuals can be reduced by giving the animals an equal treatment. Temperature especially, is an important factor, and the animals therefore were were kept at a constant temperature throughout the period of research. This meant that a number of animals lived for years at a temperature of 20°C.

Before the pieces of sponge are used for sampling, they are moistened by a HCl solution of pH 4.7. After the sponge is removed from the stomach, the gastric juice obtained is titrated with one hundredth normal sodium hydroxide taking pH 4.7 as an endpoint. The following procedure was adopted.

After weighing the dry sponge it is moistened and weighed again. The quantity of absorbed liquid is now known. The sponge is then inserted into the stomach for a predertermined length of time. Next it is weighed again to measure the quantity of secreted juice. Then it is squeezed out in 3 ml. of water of pH 4.7. One ml. of this mixture is taken and 1 drop of bromcresol blue is added. Titration is performed against a standard colour (acetate buffer pH 4.7, bromcresol blue and some glue in order to obtain a slight turbidity). The result has to be corrected for the total volume of the juice sampled. The resulting figure corresponds to the number of m. equiv. of "free" HCl absorbed by the sponge. Titration against phenolphthalein gives the value for "total" acid. The difference between the values for free and total acid represents the combined acid, and this provides a measure for the neutralizing capacity of the juice sample. I did not investigate the nature of the buffering substances which combine with the gastric acid. They probably originate from different sources. Obviously a part at least of the secreted acid is neutralized by gastric mucus, as cloudy (visible mucus) and viscous gastric juice samples have always a low acidity. On the other hand clear juice also possesses a certain acid-binding capacity (dissolved mucin). (See SIPLET *et al.*, 1948; GLASS and BOYD, 1948, 1952; GLASS, 1953).

7. Operation techniques.

a) Double vagotomy.

The technique of CROMBACH *et al.* (1958) was used. A urethane anaesthetized frog is placed on its belly. With scissors a median incision is made through the dorsal skin, running from the line connecting the caudal margins of the suprascapulae to the line which connects the hind parts of the eyes. Perpendicular to the most caudal

part of this incision a second one is made which reaches the lateral branches of the two arteriae cutaneae magnae; these branches have to remain intact. Now two triangular flaps of skin can be laid aside in such a way that the suprascapulae become visible. To lift the shoulderblade, a cut is made at its caudal margin with small scissors through the musc. latissimus dorsi. The entire median margin is freed and also a part of the foremargin (carefully, to avoid damaging of the outcoming lateral branch of the cutaneous artery). The shoulderblade can then be lifted a little. The muscles which are attached to its ventral side are cut with the aid of scissors. Next the shoulderblade is raised perpendicularly. By means of a bent probe the musc. petrohyoideus post. III is drawn upwards. At the dorsal side of this muscle run two bloodvessels: the chief cutaneous artery and the interior carotid artery (see *ECKER-GAUPP* II, 1899, Figs. 42 and 82). Between these vessels two vagus branches are situated; the larger is cut with the aid of a fine iris knife. This branch contains fibres supplying heart, lungs and stomach. By severing this branch the aim of the operation has been attained. The shoulderblade is replaced, and the dorsal scapular muscle is sewn to the musc. latissimus dorsi and the fascia dorsalis. Finally the skinpatches are sewn in their original position. The operated animals are placed in running tapwater in order to recover.

b) Section of the coeliac nerve.

The stomachal branch of the splanchnic nerve is easily reached from the ventral bodyside. The anaesthetized animal is placed on its back. With scissors a longitudinal section is made in the skin about 5 mm. to the left of the median. If the skin is pushed aside, the abdominal vein, running in the median, and the rostrad branch of the cutaneous vein, more laterally situated, become visibel. Midway between these veins and parallel to them an incision is made. The stomach and a small portion of duodenum are then pulled through the opening and placed on the right side of the belly. To prevent contact of the organs with the secretion of the skin, the animal's skin is covered by cotton wool moistened with Ringer's solution. If the stomach is gripped just below the oesophagus and approximately 1 cm. caudal of the pylorus, a loop of the intestine can be pulled out. In the mesenteria of this loop the pancreas and a number of bloodvessels are visible. Sometimes, especially in spring, it is necessary to extirpate an ovary in order to gain access to the coeliac nerve. (Only female animals were used.) By means of pointed forceps, the covering membrane (lamia mediastinalis) is ruptured.

Through the gap the region where the common intestinal artery branches into anterior mesenteric artery and coeliac artery is attainable. These vessels run in ligaments of the mesogastrium (see ECKER-GAUPP III, 1904, Fig. 92). The coeliac nerve which runs along the coeliac artery is transected by means of an iris knife close to this bifurcation. (In Fig.1 this place is indicated by an arrow.) After the organs have been replaced the muscle layer and the skin are successively closed with stitches.

Both operations described above can be performed with practically no loss of blood, and the wounds heal rapidly. Mortality following the cutting of the coeliac nerve is almost nill. Double vagotomy is a more drastic operation, although the chances for the animal's survival are still good. Once the wounds have healed, operated frogs may live for years.

CHAPTER III. REGULATION OF PEPSIN SECRETION.

8. "Spontaneous" secretion.

According to SMIRNOFF (1922) gastric secretion is only evoked if there is food in the stomach. (The article of SMIRNOFF is known to me only from a summary by PETROFF.) FRIEDMAN (1937) observed that during late spring and in summer, secretion of both acid and pepsin is spontaneous and continuous. During winter spontaneous secretion comes to a standstill. From my observations "spontaneous" secretion appears to be negligible in comparison with secretion evoked by mechanical stimulation of the stomach wall.

To reconfirm this the stomachs of frogs which had been starved for at least five days were drained by means of a moist sponge. In other frogs the stomach was ligated below the pylorus, and some days afterwards the stomach was drained. Also in decerebrated animals, and in frogs with denervated and ligated stomachs, the spontaneous secretion was measured (see Table 4).

Table 4. Pepsin output of animals treated in different ways, with and without mechanical stimulation.
(Tables I, XXVIII and XXIX.)

type of experiment	P.U.	number of expts.
intact frogs; stomach drained	1.0 ± 0.5	21
stomach ligated and drained after two days	2.9 ± 1.8	14
decerebrated; stomach ligated and drained after 2 to 4 hours	0.8 ± 0.7	7
stomach denervated and ligated; drained after two days	2.0 ± 0.9	13
intact frogs mechanically stimulated for 2 hours	26.0 ± 7.3	24
the same, for 4 hours	56.0 ± 13.4	20

In all these experiments only traces of pepsin could be detected. It is questionable whether any spontaneous secretion occurs at all, as the gastric juice gathered in the first four sets of experiments

may have been what remained in mucus and in folds of the stomach wall. The pepsin output in these four experiments is approximately of the same magnitude as the secretion of animals in which the c.n.s. has been destroyed and in which gastric secretion is mechanically stimulated (3.0 P.U.). (See Table 7.)
Since FRIEDMAN experimented with animals in which the c.n.s. has been partly or totally destroyed "spontaneous" secretion must have played an important role in his observations. As I avoided the use of pithed animals, my standard of good secretory output was of such a large value that the secretory rate of the empty stomach can be considered negligible. Because of the small outputs which FRIEDMAN obtained in his animals, residual gastric juice left in the folds of oesophagus and stomach could have interfered with his results.

9. Secretion of partly or totally extrinsically denervated oesophagus and stomach.

The controversy over nervous control of secretion has already been discussed in Section 1. SMIRNOFF (1922) and FRIEDMAN (1934) are of the opinion that gastric secretion in the frog is under sympathetic control. CROMBACH *et al.* (1958), on the other hand, were able to evoke secretion by mechanical stimulation of the stomach in sympathectomized frogs. All investigators agree that the vagus has no role in the regulation of gastric secretion. In the experiments of CROMBACH *et al.*, the nervous connection between sympathetic ganglia and stomach was left intact. The possibility that a reflex arc passing through the sympathetic ganglia is involved in gastric secretion evoked by mechanical stimuli cannot be overlooked.

In this study, sympathectomy is performed by severing the coeliac nerve, as described in Section 7. Three types of experimental animals are used. a) Intact frogs, b) animals in which the stomachs are totally deprived of their sympathetic innervation, c) vagotomized frogs in which the coeliac nerve has been cut, i.e. the entire extrinsic nerve supply of oesophagus and stomach had been eliminated. Even in the last case the reactions upon mechanical stimulation of the stomach wall are not abolished (Table 5.).

In Table 5 the secretory response of the experimental animals is compared with the "spontaneous" secretion (cf. Section 8). It appears that denervated stomachs still respond to mechanical stimulation. It is doubtful if there is any superimposed regulation by the

Table 5. Pepsin secretion of intact frogs, and of frogs with totally or partly extrinsically denervated stomachs. r.s.s. = 2; secretory period: 2 hours. (Tables I, II, III and XXVIII.)

type of experiment	pepsin outout in P.U.	number of expts.	mean weight in grams
intact frogs	26.0 ± 7.3	24	51
coeliac nerve cut	20.7 ± 6.1	20	62
vagotomy and coeliac nerve cut	15.4 ± 5.6	20	64
"spontaneous" secretion	1.0 ± 0.5	21	45

sympathetic system, as the differences between the first two sets of experiments of Table 5 are not statistically significant ($t = 1.63$; $P > 0.05$). Secretion evoked by distension of the stomach is not prevented by vagotomy, but in this case there is a significant difference between the results with intact frogs and vagotomized frogs ($t = 2.66$; $P < 0.01$).

Finally it is of special interest to investigate the influence of the intramural stomachal nerves. In Sections 12 and 14 the influence of the intrinsic nerve plexus upon secretion will be discussed.

10. **Reflex secretion.**

SMIRNOFF (1922) investigated gastric secretion in frogs provided with a fistula. From his results he concluded that in *Rana* a cephalic phase is lacking. According to SMIRNOFF the secretion depends on sympathetic control. Vagotomy has no inhibiting effect upon gastric secretion. FRIEDMAN (1934, 1937) also states that the sympathetic system regulates gastric secretion, especially acid secretion. He emphasizes the following points.

1) Vagal stimulation does not promote secretion.
2) Atropine and adrenaline both stimulate acid secretion.
3) Acid secretion is not augmented by pilocarpine and acetyl choline.
4) Stimulation of the periferal stump of the cut splanchnic nerve causes secretion of acid.
5) Mobile frogs secrete gastric juice, but after splanchnectomy activity does not influence secretion.
6) Stimulation of the central end of the sciatic nerve causes contractions of the stomach; this effect is abolished by destruction of the spinal cord, or by cutting the splanchnic nerve.

WOLVEKAMP and TINBERGEN (1942) also found that a secretory reflex via the vagus does not exist. Optical as well as gustatory stimuli failed to evoke pepsin secretion; secretion could only be elicited by mechanical stimulation of the stomach wall. Eliminating the sympathetic supply by cutting the rami communicantes 5, 6, 7 and 8, and severing the sympathetic chain between ganglia 4 and 5, and between 8 and 9, does not abolish secretion caused by mechanical stimuli. While FRIEDMAN concludes that activation of secretion is not a local affair, CROMBACH *et al.* (1958) are of the opinion that at least the start of gastric secretion is a local affair. They think that a simple mechanical stimulus applied to the stomach wall stimulates the glands possibly by means of a periferal nervous mechanism acting through sympathetic ganglia, or through the nervous plexus of the stomach wall. From the results given in Section 9, it appears that elimination of sympathetic ganglia by severing the coeliac nerve does not abolish mechanically induced secretion. Accordingly we should investigate the function of the nervous plexus in the stomach wall.

Although the normal reaction of frogs with cut coeliac nerves provides a strong argument against the opinions of SMIRNOFF and FRIEDMAN, I performed other experiments to obtain more information concerning the absence or presence of sympathetically induced reflex secretion. For this purpose, three different series of experiments were performed.

a) In Nembutal anaesthetized frogs in which the stomachal innervation was left intact, the stomach was ligated just below the pylorus. The stomach was drained in order to remove any gastric juice possibly present. The abdomen was opened, and a small mica-plate was placed between splanchnic nerve and arteria intestinalis communis. The splanchnic nerve was then electrically stimulated with a small induction coil for two hours at a frequency of one hundred stimuli per minute. At the end of the second hour, the stomach was drained again. A small gastric output was observed. On the other hand, the output was also small when the stomachs of Nembutal anaesthetized frogs were stimulated mechanically (r.s.s. = 2). Apparently this anaesthetic exerts an inhibitory effect upon secretion which obscures a possible splanchnic influence. (See Table 6.) I want to draw attention to the fact that conscious frogs show approximately 5 times as high a pepsin output as that of Nembutal treated animals when both receive mechanical stimulation (r.s.s. = 2 for 2 hours).

b) In frogs anaesthetized with Nembutal the stomach was ligated at the pylorus and the contents were removed by drainage. The sciatic

Table 6. Pepsin output in Nembutal anaesthetized frogs. Duration: 2 hours. (Tables I and XXVIII.)

type of experiment	P.U.	number of expts.
mechanical stimulation (r.s.s. = 2)	5.3 ± 2.5	19
stimulation of sciatic nerve; stomach drained	0.9 ± 0.4	4
splanchnic nerve stimulated (r.s.s. = 2)	5.4 ± 1.2	4
stomach drained	1.0 ± 0.5	21
conscious frogs; mechanical stimulation (r.s.s. = 2)	26.0 ± 7.3	24

nerve was exposed and electrically stimulated for two hours. The stomach was then drained again. No difference in output was found between the first and the second draining. The possibility that the negative results were due to an inhibiting effect of the anaesthetic was investigated by placing a sponge in the stomach of narcotized frogs, and stimulating the sciatic nerve. Here too secretion was negligible small. The experiments described above are not conclusive because Nembutal exerts a strong inhibiting effect on gastric secretion.

Nembutal (and other barbiturates) exerts a depressor effect on the cerebro-spinal system; it affects all segmental levels. Large doses suppress the vasomotor centre (GOODMAN and GILMAN, 1955). This causes a decrease in the bloodflow (CONSTANT, *et al.* 1949; SLECHTA and CALLAHAN, 1960), which in turn may be the cause of a decrease in secretory rate.

For this reason another series of experiments was performed.

c) In a urethane anaesthetized frog the pylorus was ligated, and the animal was then allowed to recover. After the stomach had been drained by means of a sponge, the animal was placed in a glass aquarium provided with a brass floor. Through this floor an electrical current was passed in pulses of ten per minute. The animals responded to each pulse with powerful leaps. In this way it was kept in a state of continuous muscular activity for two hours. At the end of these two hours the stomach was drained once more. No difference was found in pepsin content of the sponge between the first and the second drainage.

In another series of experiments mechanical stimulation of the

stomach (r.s.s. = 2) was supplied concurrently with electrically stimulated activity. After two hours a substantial pepsin yield was obtained. This secretion was smaller than the output of frogs at rest ($t = 2.56$, $P < 0.01$). (Fig. 3) From these results it is evident that there is no indication of reflex secretion via a sympathetic pathway;

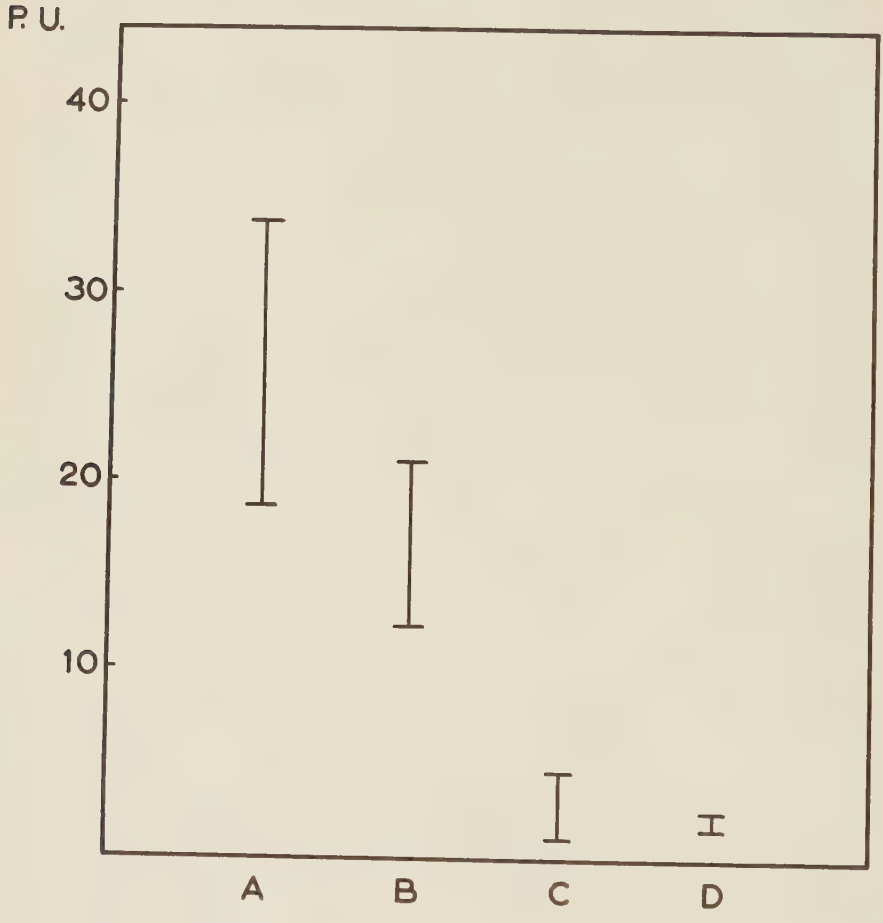

Fig.3. Influence of muscular activity on pepsin secretion.
A : stomach mechanically stimulated (r.s.s.= 2) for 2 hours.
B : the same, but the animals were compelled to jump.
C : stomach drained at the beginning of the experiment.
D : empty stomach drained after the animal had been jumping for 2 hours.
(Tables I, XXIX and XXX.)

the opinion of FRIEDMAN that jumping or roughly handled frogs show a higher secretory rate than quiet frogs could not be confirmed. On the contrary, when both groups receive mechanical stimulation of the stomach, it appears that jumping frogs have a lower secretory rate than animals which are not alarmed. This suggests an indirect sympathetic influence by regulation of the circulation. High activity of the skeletal muscles may lead to a decrease in bloodflow to the digestive tract. Sympathetically induced vasoconstriction in the splanchnic area might inhibit gastric secretion. If so, we have to consider the influence of circulatory adjustments on gastric secretion. This point will be discussed in Section 13.

11. Gastric secretion in frogs with damaged central nervous system.

As was mentioned previously, FRIEDMAN (1937) concluded from his investigations concerning pepsin secretion in *Rana esculenta* L. that this secretion is under sympathetic control. He used animals in which the c.n.s. was partly or totally destroyed by pithing either the brain, the spinal cord, or both. According to this author (1934), destruction of the brain alone does not change the secretory response to mechanical stimulation. Since yohimbine lowers the bloodpressure but leaves the secretion unchanged, FRIEDMAN believed that a lowered bloodpressure resulting from destruction of the c.n.s. could not cause a decrease in secretion. As secretion stopped after pithing of the spinal cord, he concluded that the rupture of a reflex arc in the spinal cord was the cause of this inhibition.

LANGLEY and ORBELI (1911) found that destruction of the c.n.s. slows the circulation, though it is not entirely stopped. Circulation is less disturbed if only the brain has been destroyed. However, there may be an important difference between lowering the bloodpressure by destroying the c.n.s., and low bloodpressure caused by yohimbine. In the latter case, low bloodpressure in the brain might be partly raised by sympathetic impulses which cause local vasoconstriction in other parts of the body. Furthermore, pithing of the brain involves a considerable loss of blood. It follows that conditions in the two experiments cannot be compared.

As we are specifically interested in the circulation in the area of oesophagus and stomach, general conclusions concerning lowering of the bloodpressure are irrelevant. Data on the rate of bloodflow in the stomach wall under different conditions would increase our insight into the role played by bloodpressure in secretory regulation. FRIEDMAN's experiment with yohimbine is not conclusive where it

concerns destruction of the c.n.s. with resultant circulatory changes allegedly influencing secretion. The results, mentioned in Section 9, show that the sympathetic system need not be intact to permit stimulation of gastric secretion. Furthermore, I did not observe any increase of pepsin output in mobile frogs. The figures shown in Table 7 clearly demonstrate the unfavourable influence of a damaged c.n.s. on pepsin secretion.

Table 7. Influence of destruction of different parts of the c.n.s. on pepsin secretion. r.s.s. = 2; duration: 4 hours. (Tables XXVII and XXVIII.)

type of experiment	P.U.	number of expts.
intact animals	56.0 ± 17.5	20
section through medulla oblongata	12.4 ± 4.0	9
brain pithed	8.8 ± 3.3	15
whole c.n.s. pithed	3.0 ± 1.6	11
intact animals; stomach drained only	1.0 ± 0.5	21

It was found that destruction of the brain causes a strong inhibition of secretion, and pithing the spinal cord further reduces secretion. If we compare pepsin secretion after mechanical stimulation in frogs with destroyed brains with the pepsin production in normal animals which were not stimulated, we find that the former react feebly to mechanical stimulation. Since, in animals with intact c.n.s. in which the stomach is deprived of its extrinsic innervation, secretion is only slightly lowered, I consider that the results mentioned in Table 7 are not caused by direct nervous influences, but indirectly by the blood circulation. The much greater decrease in secretion following pithing of the spinal cord may be the result of the destruction of vasomotor centres, or of loss of blood, or of both. Referring again to the effect of Nembutal anaesthesia previously discussed, the suppression of the central vasomotor activity by Nembutal causes a decrease in secretory rate which is comparable to the decrease occurring in animals with a destroyed brain (cf. Table 6).

Some results obtained by CROMBACH *et al.* (1958) also show that destruction of the c.n.s. reduces the secretory output. (Table 8). My findings in general agree with those of CROMBACH *et al.*

Table 8. Pepsin output in frogs with destroyed brain, and in sympathectomized frogs.(Data assembled from CROMBACH *et al.*, 1958. The digestion percentages given by CROMBACH are here converted into pepsin units.)

type of experiment	P.U.
c.n.s. pithed; stomach not stimulated	1.5
c.n.s. pithed; stomach stimulated	9.1
c.n.s. intact; sympathectomized stomach	42.4

12. Pharmacological inhibition of the regulation of pepsin secretion by the autonomic nervous system.

From the experiments mentioned in Section 9, it appeared that cutting the extrinsic nerves which run to stomach and oesophagus does not prevent gastric secretion. The next question is whether the nervous plexus in the stomach wall exerts any regulatory influence on gastric secretion. The intramural plexuses can not be removed by surgery, and the system was therefore studied with the aid of drugs. Three different drugs were used : dihydro-ergotamine (DHE), hexamethonium chloride (C6), and atropine sulphate.

DHE abolishes the pressor effect of sympathetic nerve stimulation and of adrenaline; it paralyses the sympathetic motor fibres, but not the inhibiting fibres. DHE is a powerful vasoconstrictor by its direct action on smooth muscles (GOODMAN and GILMAN, 1955). According to LOEWI and NAVRATIL (1926) ergotamine has a sensibilizing effect on the vagus in *Rana* : the heart becomes more sensitive to vagal stimuli. My observations on the frog's heart show that subcutaneous injection of 4 mg. DHE per kg. bodyweight does not change the heart rate, but the stroke volume decreases.

C6 has a vasodilatory effect which results in a lowering of the arterial bloodpressure. Blocking is brought about by raising the threshold of the ganglion cell for acetyl choline liberated by the preganglionic nerve. Accordingly the impulse conduction in the autonomic system is inhibited. The impulse transmission to the skeletal muscles is blocked too. The dose I used (50 mg. of C6 per kg. bodyweight) totally paralysed the frogs. Paralysis lasted 8 hours in most cases. The heart rate was found to be unchanged. The inhibitory effect of vagal stimulation on the heart, however, was abolished. This is illustrated in the following protocol :

In a spinal frog the heart and the left vagus were exposed. The heart rate was counted. Next the vagus was electrically stimulated for a short time. Successively the following figures were obtained:
heart rate: 57 beats per minute, vagal stimulation: standstill of the heart after 3 beats; heart rate: 52 b.p.m., vagal stimulation: standstill after 3 beats; heart rate: 47 b.p.m., vagal stimulation: standstill after 5 beats; heart rate: 44 b.p.m. Next, C6 was injected intraperitoneally. After 25 minutes the heart rate was 48 b.p.m., and during stimulation of the vagus 41 b.p.m. After the stimulation was stopped the heart rate remained 41 b.p.m. Later the heart rhythm became irregular. The slight decrease in heart rate during the following period of vagal stimulation (36 b.p.m.) must be ascribed to a general decay in the condition of the heart, for at the end of the experiment (1¼ hour after the beginning) the rate had slowed down to 32 beats per minute.

From this protocol it appears that after injection of C6 stimulation of the vagus no longer results in a standstill of the heart.

Atropine exerts a selective blocking effect on effectors which are innervated by postganglionic cholinergic nerves. The block takes place at the site of the effector cells, no matter whether the innervation is intact or not (GOODMAN and GILMAN, 1955). Atropine has no marked effect on vascular reactions or on bloodpressure. The dose I used amounted to 20 mg. atropine per kg. bodyweight. In order to check the effectiveness of the drug, its action on heart activity was tested. Following atropine injection the heart rhythm remained unaltered, but the stroke volume increased. The following two protocols relate to the effect of atropine injection.

An intact frog was tied on its back on a 2 litre bottle. Under a favourable illumination the heart beats could be observed through the skin, which was stretched taut by this method of fettering. Four successive counts gave 61, 62, 59 and 60 beats per minute (temperature 22° C.). Then the animal was injected with atropine sulphate. During the next 2.5 hours there was no change of the rhythm, though the beats seemed to be more powerful.

In another frog the heart rate amounted to 42, 36, 42, 44 and 43 beats per minute (temperature 18° C.). 20 minutes after atropine injection it was still 43 b.p.m., after 30 minutes: 41 b.p.m., after 45 minutes: 44 b.p.m. After pithing the c.n.s.: 47 b.p.m. 30 minutes later: 34 beats per minute. The heart and the vagus were then exposed. One hour later the heart rhythm amounted to 37 beats per minute. Stimulation of the vagus did not change this rhythm. The whole experiment lasted 2 hours and 45 minutes.

It is shown that after atropine injection vagal inhibition is abolished.

In the experiments on secretion the injections were given into the ventral subcutaneous lymph sac. For this purpose the syringe was pushed through the skin of the thigh into the femoral lymph sac, and from there through the inguinal septum into the abdominal lymph sac. In this way loss of injection fluid through the hole in the skin is prevented.

Pepsin secretion was evoked by means of a sponge (r.s.s. = 2)

which remained in the stomach for 2 hours. Animals injected with Ringer's solution (28 ml.per kg. bodyweight) acted as controls. The results of these experiments are collected in Table 9 and represented in Fig. 4.

Table 9. Pepsin secretion in P.U. of intact frogs, and of frogs with partly or totally denervated oesophagus and stomach, after administration of several drugs. Mechanical stimulation during two hours. 277 experiments. (Tables I, II and III.)

type of experiment	Ringer	DHE 4	DHE 0.78	C6	Atropine
intact animals	26.0 ± 7.5	21.6 ± 7.3	24.4 ± 8.2	18.8 ± 5.0	14.8 ± 3.7
coeliac nerve cut	20.5 ± 5.6	16.9 ± 4.1	27.8 ± 7.9	14.0 ± 5.6	9.9 ± 2.6
vagotomy, coeliac nerve cut	15.4 ± 5.6	16.2 ± 4.5	---	7.0 ± 2.6	10.2 ± 1.8

The secretory rate was scarcely changed by DHE. The fact that secretion is not diminished suggests that sympathetic secretory fibres, if present, do not play a role in the regulation of secretion. Changes in the rate of bloodflow might influence secretion, but since there is no change in the secretory output, we may assume that this possibility is not realised. It is true that DHE inhibits the activity of the sympathetic vasoconstrictor nerve fibres, but on the other hand DHE does have a constrictor effect through its direct action on the smooth muscles of the bloodvessels. These two effects may cancel each other, and this makes it difficult to draw any conclusions concerning the effect of DHE on the bloodvessels. These experiments, however, suggest that there are no direct sympathetic influences on pepsin secretion.

The lower value of the mean pepsin output obtained after administration of C6 is not statistically significant. C6 may have a twofold influence on gastric secretion. The vasodilator effect will create a vascular condition favourable for secretion, whilst blocking of the autonomic impulses would inhibit secretion elicited by the activity of extrinsic nerves. These two antagonistic tendencies raise difficulties in the interpretation of the results. C6 is a strong inhibitor of gastric secretion in man (KAY and SMITH, 1950,1956), and it exerts its effect by blocking the vagus. In *Rana* this effect is weak.

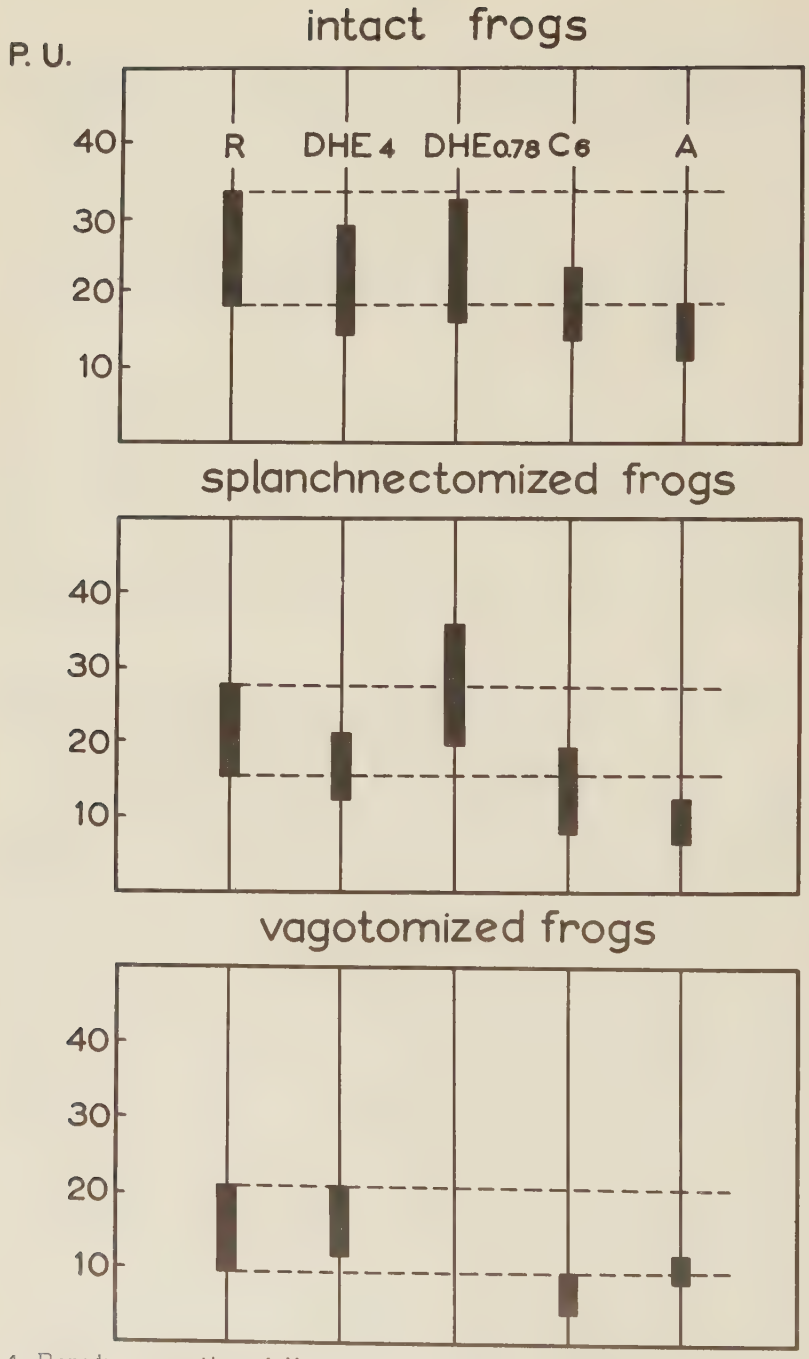

Fig.4. Pepsin secretion following subcutaneous injection with Ringer's solution (R), a large dose of dihydro-ergotamine (DHE 4), a small dose of dihydro-ergotamine (DHE 0.78), hexamethonium chloride (C6), and atropine sulphate (A). r.s.s. = 2; secretory period 2 hours.

Atropine causes a marked inhibition of secretion. Since this drug hardly changes the circulation the lowering of the secretion can be ascribed to the blocking of parasympathetic secretory fibres.

The experiments with DHE, C6 and atropine lead to the conclusion that the pepsin secretion is regulated by cholinergic fibres of the intramural nerve plexus, whilst no direct regulation by the sympathetic components in this plexus was found.

13. Vascular reactions and pepsin secretion.

In the present Section the influence of blood supply on secretion will be discussed, and supplemented with the results of some experiments. It has already been pointed out that destruction of the c.n.s. strongly inhibits gastric secretion (see Section 11). Starting from the observation of LANGLEY and ORBELI (1911) that destruction of the c.n.s. in *Rana* interferes with circulation, I ascribed the large decrease in secretion to vascular influences and not to blocking of a reflex path, because cutting the extrinsic stomachal innervation inhibits gastric secretion only to a minor degree.

Nembutal also slows down the rate of bloodflow. In the frog, weakening of the circulation and a strongly inhibited secretion occur together after administration of this anaesthetic.

Other evidence that a diminished blood supply to the foregut reduces the secretory rate has been provided by the reduced pepsin output of frogs which are kept moving continuously (see Section 10). It is to be expected that the high adreno-sympathetic tone of these animals exerts a vasoconstrictor effect in the splanchnic area. This vasoconstriction would lead to a lowering of gastric secretion, which, in fact, was found.

In general, dilation of the vessels not only increases the quantity of blood flowing through the organs, but it also favours the exchange of metabolic products between blood and tissues as a result of the decreased rate of flow.

BABKIN *et al.* (1939) provided evidence that the sympathetic nerve supply to the cat's pancreas contains cholinergic secretory fibres. After stimulation of the splanchnic nerves by means of an induction coil the venous blood of the pancreas contained acetyl choline (proof with an eserinized leach muscle). The secretory activity of the pancreas is predominantly parasympathetically controlled, but both cholinergic stimulation of the splanchnic nerve and vagal stimulation can cause secretion.

KUNTZ and RICHINS (1949) gave proof of the existence of adrenergic sympathetic fibres, which after being stimulated cause a decrease in the secretory rate. Previously RICHINS (1945) had

discovered that all sympathetic fibres, which innervate the pancreas, end in the wall of bloodvessels. Direct sympathetic innervation of the glands was not observed. This means that pancreatic secretion following sympathetic stimulation results from a vasomoter reaction. RICHINS (1953) worked with Nembutal anaesthetized cats in which the vagi were cut below the diaphragm. The cholinergic nerves were paralysed with the aid of atropine so that stimulation of the splanchnic nerve resulted in adrenergic stimulation. In another series of experiments, the adrenergic fibres were inactivated by means of ergotoxine so that splanchnic stimulation resulted in cholinergic stimuli. To be able to observe the vascular pattern at a given time, the pancreatic tissue was rapidly frozen in situ by means of a mixture of dry ice and acetone. Next the tissues were fixed using the freeze-drying method (RICHINS, 1948). The diameter of the bloodvessels was measured in the sections. Adrenergic sympathetic stimulation appeared to cause vasoconstriction in the pancreas, and this accompanied a decrease in secretory rate. Cholinergic sympathetic stimuli resulted in vasodilation, which was paralleled by a rise in secretion. In this way RICHINS demonstrated that pancreatic secretion is also under sympathetic control, i.e. through vasomotor reactions.

A comparable result has been obtained with the salivary glands of several mammals. KUNTZ and RICHINS (1946) found with the aid of a degeneration method that the sympathetic fibres of the salivary glands mostly supply the bloodvessels, while the innervation of the glandular cells is mainly parasympathetic. In 1953 RICHINS and KUNTZ demonstrated that the secretory activity of the parotid gland is regulated by parasympathetic fibres, and that there exists a sympathetic influence on the secretion which is effected through vasomotor fibres.

The investigations of RICHINS and KUNTZ provide several examples of an indirect influence of sympathetic nerves on the secretory activity of digestive glands. The results of the following experiments on the frog extend and, in a certain way, confirm the idea that in vertebrates secretion is influenced by this indirect sympathetic effect.

In view of the strong inhibition of gastric secretion in decerebrated or anaesthetized frogs, the experiments in which the influence of alterations of the bloodpressure is examined were performed on conscious animals. In order to obtain changes in bloodpressure, sodium nitrite was used. Nitrite causes relaxation of smooth muscles, especially of those of the smaller bloodvessels. The relaxed muscles are not paralysed, and motor nerve impulses keep their constrictor effect (GOODMAN and GILMAN, 1955). Injections were given into the ventral lymph sac; the dose amounted to 15 mg. per kg. bodyweight. Gastric secretion was evoked by placing a sponge (r.s.s. = 2) in the stomach for two hours. Frogs which were injected with Ringer's solution (28 ml. per kg.) acted as controls. The results of these experiments are presented in Table 10 and in Fig. 5.

Sodium nitrite appears to inhibit the gastric secretion of intact frogs. This seems strange at first because one expects vasodilation to exert a favourable influence on secretion. On the other hand, the fact that the smooth musculature of the bloodvessels retains its

Table 10. Pepsin secretion (P.U.) of intact frogs and of splanchnectomized frogs, after subcutaneous administration of respectively Ringer's solution, a parasympathomimeticum, a vasodilatory substance, and a combination of both. r.s.s. = 2. secretion period: 2 hours. 160 experiments. (Tables I and II.)

experimental animals	Ringer 28 ml/kg	atropine 20 mg/kg	nitrite 15 mg/kg	atropine + nitrite
intact frogs	26.0 ± 7.5	14.8 ± 3.7	11.8 ± 3.1	10.3 ± 2.6
splanchnectomized frogs	20.5 ± 5.6	9.9 ± 2.6	29.9 ± 8.2	18.6 ± 6.3

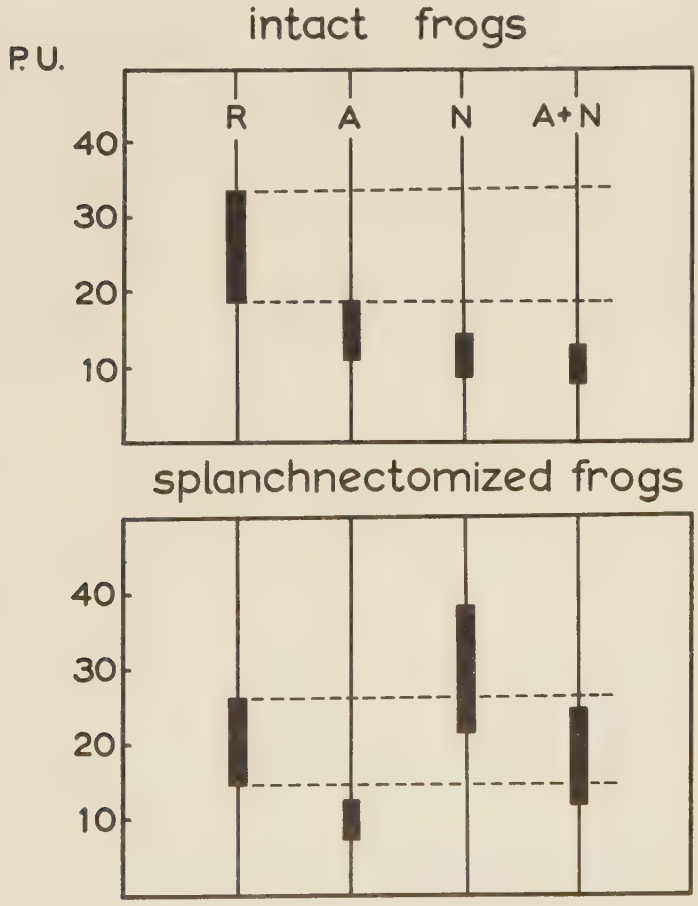

Fig.5. Pepsin secretion following subcutaneous injection with Ringer's solution (R), atropine sulphate (A), sodium nitrite (N), atropine sulphate and sodium nitrite combined (A + N). r.s.s. = 2; secretory period 2 hours.

sensitivity to vasomotor impulses offers a possible explanation of this inhibition, for sympathetic impulses cause constriction of the bloodvessels in the splanchnic area. Vasoconstriction in the bowels, by shifting part of the blood volume, may counteract lowering of the bloodpressure in brain and skeletal muscles which would otherwise accompany the general depression in bloodpressure caused by nitrite. If this is true, cutting of the sympathetic nerves followed by the administration of nitrite, which causes vasodilation, should result in a copious flow of gastric juice.

In point of fact, when the secretion of intact animals and of frogs in which the coeliac nerve has been cut is compared after administration of nitrite, a marked difference is found. Operated frogs produce more than two and a half times as much pepsin as intact animals ($t = 9.82$; $P < 0.001$). This significant effect is an example of the extent to which the circulation is able to regulate pepsin secretion. In intact frogs the unfavourable vascular conditions decrease the output even more than does blocking the parasympathetic secretory fibres by atropine. On the other hand, the inhibitory effect of atropine can be counteracted by nitrite in splanchnectomized frogs. If a combined atropine and $NaNO_2$ injection is given to such frogs, an output is obtained which is intermediate between that obtained after separate nitrite or atropine injection. Following the combined injection, secretion reaches the level of the control (see Fig. 5).

CHAPTER IV. REGULATION OF ACID SECRETION.

14. Neurogenic regulation.

From the experiments described in the previous Chapter it appears that the sympathetic system does not exert any direct influence on pepsin secretion, this secretion being mainly under parasympathetic control. Double vagotomy causes a significant though not excessive lowering of the pepsin output, and parasympathetic blocking with the aid of atropine brings about a comparable lowering (Table 9).

FRIEDMAN (1934) found that vagal stimulation in *Rana* failed to elicit an acid secretion whereas atropine augments the secretion of acid; stimulation of the splanchnic nerve or administration of adrenaline raises the acid output. Since, according to FRIEDMAN, destruction of the brain does not inhibit secretion, whilst pithing of the spinal cord does, he concludes that "gastric secretion is probably not due to an axon reflex but to a true reflex involving afferent paths from the stomach along the sympathetic nervous system to the region of the cord giving off spinal nerves 2 to 5 (= *3 to 6 in accordance with* GAUPP's *numbering*) and from there back through secretory efferent paths also in the sympathetic nervous system".

SMIRNOFF (1922) also found that vagotomy does not exert an inhibitory effect on gastric secretion.

In discussing the regulation of pepsin secretion in Section 9 it was proposed that cutting the sympathetic stomachal nerve does not significantly lower the secretory response to mechanical stimulation of the stomach wall. It is true that double vagotomy causes a decrease of the secretory output, but in vagotomized frogs a secretory response of considerable magnitude to mechanical stimuli still exists (Table 5).

My experiments on acid secretion were planned on the same basis as my experiments on pepsin secretion. Some results previously obtained by KLOK and SMIT (in press) must be summarized first while the technique we used will be described in detail.

Again, pieces of sponge were used to evoke gastric secretion. Since the acid secreting glands are situated in the pyloric part it is important to push the sponge to the bottom of the stomach. After an appropriate time the sponge is removed and the collected gastric juice is measured according to the method described in Section 6. In order to test whether the absorption capacity of the sponge was great enough, sponges were examined after remaining for varying

periods of time in the stomach. From Fig. 6 it is evident that the quantity of gastric juice in the sponge increases with increasing time, and that, if the duration of the experiments does not exceed 6 hours, the absorption capacity of the sponge is sufficient. In most experiments length of time the sponge remained in the stomach did not surpass 4 hours, and the limit of absorption was therefore not reached.

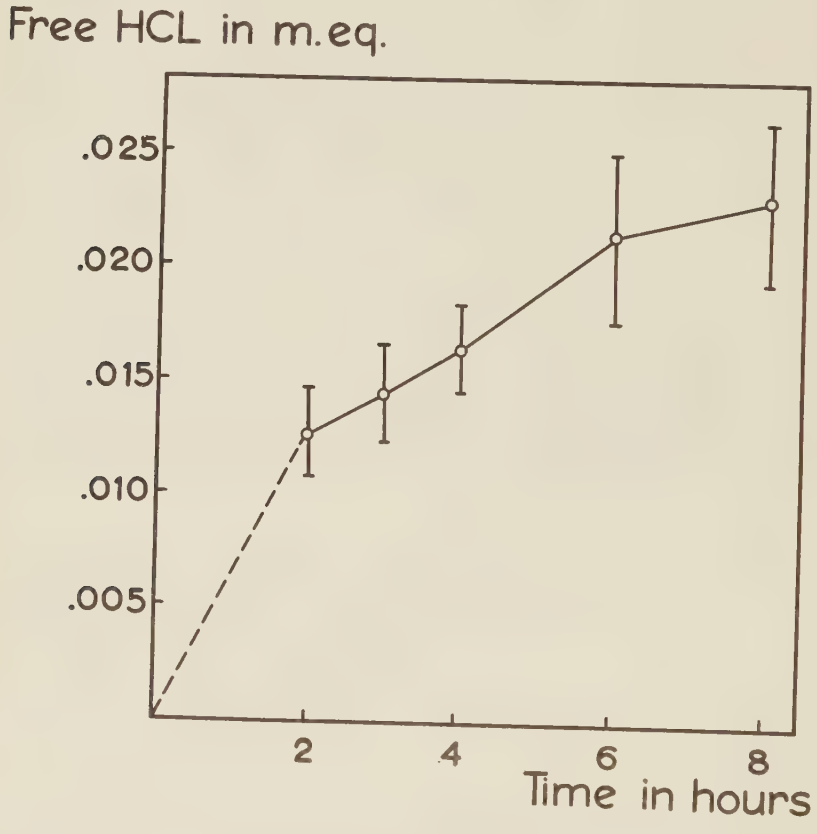

Fig.6. Acid output in relation to length of time the sponge was in the stomach (r.s.s. = 2).

2 hours : 0.0128 ± 0.0020 m.eq. HCl
3 hours : 0.0145 ± 0.0022 " "
4 hours : 0.0164 ± 0.0020 " "
6 hours : 0.0215 ± 0.0036 " "
8 hours : 0.0229 ± 0.0036 " "

(After KLOK and SMIT, in press)

For measurement of acid secretion, as for pepsin secretion, the accurate regulation of mechanical stimulation was important. KLOK and SMIT report the following figures. The output of free HCl during a 4 hours secretion period amounted to 0.0081 ± 0.0017 m. equiv. of HCl if secretion had been evoked by a small sponge (r.s.s. = 1). Using a larger sponge (r.s.s. = 3) the free HCl output amounted to 0.0127 ± 0.0021 milli-equivalents. Thus for acid secretion too, a larger sponge causes a greater output.

If secretion is the result of a reflex via the sympathetic system, it should be possible to inhibit secretion by interrupting the reflex path. As was expected, pithing the animals proved to be an unsatisfactory method (see Section 11). This is illustrated by the results obtained by KLOK and SMIT, who measured free HCl output of frogs. Secretion was evoked by a sponge (r.s.s. = 2) which remained in the stomach for 4 hours. Intact frogs yielded 0.0164 ± 0.0020 m. equiv. of HCl. After pithing the brain the output decreased to 0.0089 ± 0.0023 m. equiv. When both brain and spinal cord were destroyed, no free HCl could be detected at all. In Section 11 it has already been pointed out that disturbed circulation must be the cause of the breakdown in gastric secretion.

KLOK and SMIT found that cutting the coeliac nerve does not affect acid secretion (Table 11).

Table 11. Gastric acid output of frogs in which secretion was evoked by means of a mechanical stimulus (r.s.s. = 2). Secretion period 4 hours. (After KLOK and SMIT, unpubl.)

type of experiment	mean output of free HCl in m.equiv.	number of expts.
intact frogs	0.0178 ± 0.0032	33
coeliac nerve cut	0.0188 ± 0.0027	33
vagotomy and coeliac nerve cut	0.0178 ± 0.0043	9

The assumption of FRIEDMAN that sympathetic nerves play an important role in the regulation of acid secretion could not be confirmed.

Vagotomy too failed to lower the secretion of acid. The nervous mechanism which controls the amount of secretion in direct proportion

to the strength of mechanical stimulation, must be sought in the intramural nerve plexus of the stomach. On the basis of these experiments, a theory of regulative influence by reflex paths which run via the c.n.s. must be denied. I also could not confirm the results of FRIEDMAN that atropine stimulated the secretion of acid. My experiments, on the contrary, proved that atropine has an inhibiting influence on the HCl output (Table 20). Thus the present results agree with those obtained in the investigation of pepsin secretion. Since vagotomy does not cause any lowering of the acid secretion whilst atropine does, it must be concluded that the secretion of acid is influenced by the regulative action of intramural parasympathetic fibres.

15. Influence of histamine on acid secretion.

In 1919 POPIELSKI discovered that subcutaneous administration of histamine to dogs elicited secretion of acid gastric juice. Since this response to histamine was also found after vagotomy and after injection of atropine, he thought that histamine probably had a direct action on the glands. Later POPIELSKI assumed histamine to be identical with his hypothetical vasodilatin. It is true that histamine has a vasodilatory action, but not every vasodilator exhibits the secretagogue effect of histamine (see a.o. LIM *et al.*, 1927; STEIGMAN *et al.*, 1952; NORDGREN and ÖBRINK, 1956). On the other hand, the vasodepressor effect of histamine can largely be reduced by using antihistaminics, while its secretagogue effect remains unchanged (ROBERTSON and GROSSMAN, 1952; KAY, 1953; GOODMAN and GILMAN, 1955; JANOWITZ and HOLLANDER, 1957).

That histamine plays a role in the normal physiology of gastric secretion is indicated by its presence in gastric juice. MACINTOSH (1938) demonstrated histamine in gastric juice of dogs in which secretion was evoked by sham-feeding, by histamine injections or by vagal stimulation. In all three cases the histamine content of the gastric juice was the same. EMMELIN and KAHLSON (1944) found histamine in the gastric juice of dogs and cats regardless of the manner in which secretion had been elicited (spontaneous secretion; sham-feeding; electrical stimulation of the vagi; application of secretagogues in the stomach; injections of eserine, "gastrin", acetyl choline and Priscol). They point out that parietal secretion under all circumstances implies the discharge of histamine. The fundic mucosa of dogs and cats contains large quantities of histamine (GAVIN *et al.*, 1933; EMMELIN and KAHLSON, 1944; FELDBERG and HARRIS, 1953), and no histaminase (BEST and MCHENRY, 1930). This histamine store in the fundus is sufficient to be the source of the histamine content of the gastric juice, even during long secretory periods. On the other hand the histamine content of the blood is much lower than that of the gastric juice. An increased histamine percentage in the blood during secretion has not been observed.

Part of the histamine is broken down in the liver. GREGORY (1958) could considerably enhance gastric secretion, evoked by feeding or sham-feeding, by diverting part of the portal bloodstream to the systemic circulation by partly tying off the hepatic portal vein. SIRCUS (1953) was able to obtain a distinct increase

in secretion evoked by insulin, carbachol, ethanol, histamine, food or by vagal stimulation, with the aid of B_1-pyrimidine (a substance which enhances the action of histamine). B_1-pyrimidine when given separately did not stimulate secretion. SCHAYER and IVY (1957, 1958) were able to show that feeding causes a decrease in the histamine content of the stomach wall of rats. This result could not be confirmed by HILL and CODE (1959) who used dogs. HILL and CODE thought that the histamine of the mucosa might have been replenished as rapidly as it was given off.

The investigations of these workers not only illustrate the stimulatory effect of histamine on gastric secretion, but they demonstrate as well that histamine plays a role in the normal physiology of gastric juice secretion acting as a stimulant for the acid secreting cells. As BABKIN says: "....histamine is a chemostimulator for the parietal cells under any conditions which lead to the secretion of gastric juice."

Reviews of the investigations and problems mentioned above are given by GROSSMAN(1950), BABKIN (1950), CODE (1951), GREGORY (1952), JAMES (1957) and IVY (1958).

Several investigators have studied the influence of histamine on gastric juice secretion in Amphibia. KEETON, KOCH and LUCKHARDT (1920) evoked gastric secretion in bull frogs by means of histamine, but they failed to obtain this result in *Rana esculenta* L. POPIELSKI (1929) was able to enhance gastric secretion in the edible frog with the aid of histamine, but only if the temperature was raised to 37°C. According to DELRUE (1933) histamine stimulates acid secretion in the isolated gastric mucosa of *Rana temporaria* L. This was confirmed by GRAY *et al.* (1940). In *Rana esculenta* L. FRIEDMAN (1937) obtained a liberal quantity of gastric juice of a high acidity after administration of histamine. On the other hand histamine failed to stimulate acid secretion in *Necturus* (FRIEDMAN, 1942).

In the following experiments the influence of histamine on the secretion of gastric juice in *Rana esculenta* L. is investigated. Since the juice was collected by means of a sponge, histamine induced secretion, if present, would be superimposed upon the flow of gastric juice caused by mechanical stimulation by the sponge. The difference between the output of gastric juice with and without histamine could be amplified by reducing the effect of mechanical stimulation with atropine sulphate. This drug was used because it blocks the cholinergic nerves which play a role in secretion, but it does not abolish the direct action of histamine on the secretory cells.

In a series of 20 animals in which sponge-induced secretion was partly inhibited by atropine, the mean quantity of free HCl after two hours amounted to 0.00551 m. equiv.; in another experiment in which atropine and histamine were administered the mean yield after two hours amounted to 0.03184 m. equiv. of free HCl, a nearly sixfold quantity.

Thus histamine has a strong stimulating effect on acid secretion.

The next experiments were performed in order to investigate the influence of histamine on the composition of the gastric juice. In a group of 15 frogs the mechanically stimulated secretion (r.s.s. = 2) was measured with and without administration of histamine. The gastric juice was sampled after 0.5 hour, 1 hour and 2 hour periods. For each of the two types of secretion, 3 series of experiments were performed representing 3 different secretory periods. Of these juice samples total acid, "free" HCl, acid binding capacity, peptic activity, and the concentrations of acid, buffer and pepsin were measured (Table 12). If the outputs of these components are plotted against time, S-shaped curves are obtained. For data on volume of secretion and output of free HCl see Fig. 7. In my opinion this S-shape is

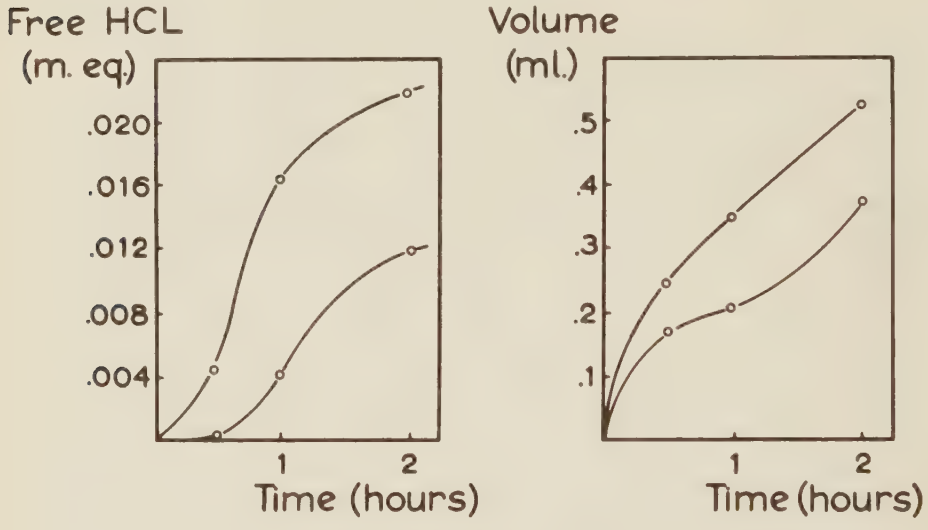

Fig.7. Output of "free HCl" and volume of total secretion plotted against time. The lowest curves refer to gastric secretion which has been evoked by means of a sponge (r.s.s. = 2). The upper curves represent secretion evoked by a sponge and by injection of histamine.

fortuitous as it depends on experimental variation. Certainly the animals have not all been exposed to the same secretory stimulus, for first, it is difficult to ascertain if the stomach wall has been stretched to the same extent in all animals, the relative sponge size which is based on the animal's bodyweight is not an exact measure

Table 13. Secretion time, output and concentrations of acid, buffer and pepsin, before and after subcutaneous histamine injection. Data classified in groups of equal secretion volume. The figures above the double lines refer to secretion which has been evoked by mechanical stimulation. The lower figures represent the secretion evoked both by mechanical stimulation and by histamine. r.s.s. = 2. Dose: 0.5 mg histamine per kg bodyweight. 90 gastric juice samples.

volume (ml.)	time (min.)	total acid (m.eq.)	free HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	total acid conc.	free HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (P.U./ml.)
0.150	53	0.00680	0.00363	0.00317	7.0	45	24	21	50
0.251	64	0.01006	0.00491	0.00515	11.6	40	20	21	48
0.483	120	0.02177	0.01132	0.01045	24.0	47	25	22	50
0.173	41	0.00843	0.00473	0.00370	7.0	49	27	22	40
0.251	47	0.01421	0.00976	0.00445	15.4	58	40	18	64
0.346	58	0.01977	0.01384	0.00593	16.4	57	40	17	48
0.452	90	0.02504	0.01746	0.00758	21.0	56	40	17	48
0.627	114	0.03509	0.02400	0.01109	29.0	57	40	18	46

(see.p.19); and secondly, there may be individual differences in sensitivity to the mechanical stimulus. Furthermore, changes in sensitivity during the secretory period could play a role. The secretion time, and thus the classification of the figures, is chosen arbitrarily and is not determined by temporal changes in sensitivity of the animal to the mechanical stimulus. It is better, therefore, to group the data according to the results, making the assumption that animals with similar responses have received equal stimulation. Measurement of the stimulus, in this context, becomes a function of individual sensitivity, temporal sensitivity, sponge size and the time of its sojourn in the stomach. The data which I grouped into classes of equal time (Table 12), I shall now classify in groups of equal secretory volumes (Table 13). If the data, classified in this way, are plotted against time, a linear relationship is found. This is true not only for the volumes, but for acid, pepsin and buffer too (Fig. 8). (The influence of histamine on pepsin secretion will be considered in Section 18

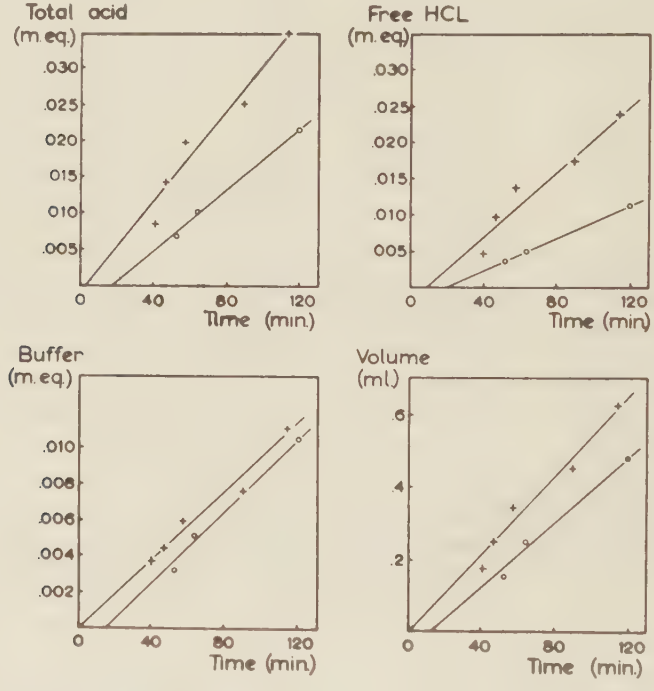

Fig.8. Secretory output of several components with and without histamine injection in relation to time. The upper lines represent the yield following histamine administration. For all lines the correlation is high ($r > + 0.80$). Statistical curves for 90 samples of gastric juice.

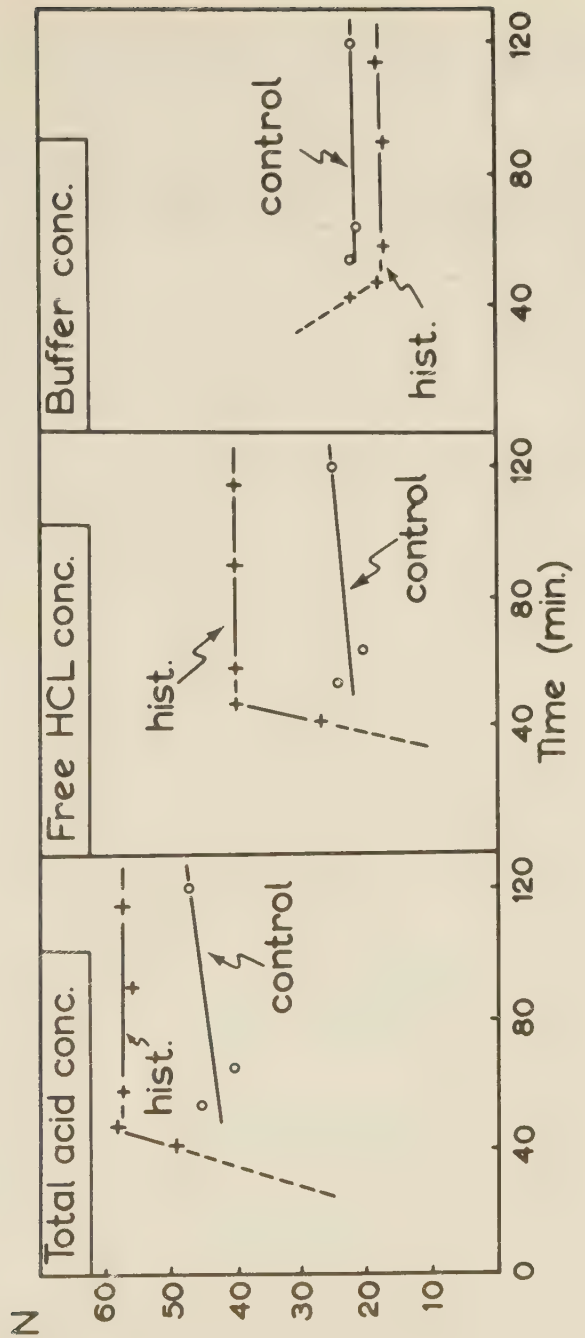

Fig.9. The relation of acid and buffer concentration in gastric juice to time. Secretion evoked by mechanical stimuli (control), and evoked both by mechanical stimuli and histamine (hist.). 90 gastric juice samples.

Table 14. Output and composition of gastric juice before and after histamine administration. Mean secretory period: 70 minutes. r.s.s. = 2. Dose: 0.5 mg. histamine-diHCl per kg. bodyweight. 90 gastric juice samples. (Tables V – XI.)

component	control	histamine	t	P
volume (ml.)	0.252 (100%)	0.377 (150%)	3.83	< .005
total acid (m.eq.)	0.01096 (100%)	0.02102 (195%)	4.04	< .005
free HCl (m.eq.)	0.00549 (100%)	0.01462 (266%)	4.44	< .005
buffer (m.equiv.)	0.00532 (100%)	0.00642 (121%)	1.63	
pepsin (P.U.)	12.0 (100%)	18.0 (150%)	2.49	< .01
total HCl conc.(mN)	44 (100%)	56 (127%)	1.80	< .05
free HCl conc.(mN)	23 (100%)	38 (165%)	2.56	< .01
buffer conc. (mN)	21 (100%)	18 (86%)	2.02	.025
pepsin conc.(PU/ml.)	48 (100%)	48 (100%)		

The lines showing the secretory response to histamine lie above those of the controls, and in the case of acid secretion the slope is steeper. Obviously temporal sensitivity to stimuli is not the same for different types of stimulation, and different components of the secretion also diverge in this respect. Histamine increases the rate of acid secretion as well as the total quantity of acid produced. The mean output of buffer is also increased, but the temporal characteristic has not been changed by histamine; the experimental and control lines run parallel. This influences the composition of the gastric juice; the increase in acid output following histamine injection is proportionally greater than the increase in volume, and the acidity of the histamine stimulated secretion is therefore higher than that obtained in the control experiment. Fig. 9 represents the concentration of acid and buffer in relation to time. The acidity is increased by histamine. On the other hand the concentration of buffer is slightly decreased.

The mean secretory output and the composition of the gastric juice with and without histamine administration are represented in Table 14.

16. **Experiments with mammalian gastrin.**

It is now generally accepted that gastric acid secretion in mammals is regulated by a hormonal mechanism. The experiments performed by IVY and his co-workers clearly demonstrated the existence of this hormonal mechanism, for they used dogs with stomach pouches transplanted subcutaneously, and thus completely denervated (IVY and FARRELL, 1925; GREGORY and IVY, 1941; BUTLER, HANDS and IVY, 1943; GROSSMAN, ROBERTSON and IVY, 1948).

The pyloric antrum functions as an endocrine organ. The release of gastrin is stimulated by the contact of food with the antral mucosa. Distension of the antrum also causes gastrin production. As local anaesthesia prevents the release of gastrin the intramural nerve plexus must play a role in gastrin release (see a.o. WOODWARD and DRAGSTEDT, 1960). Vagal impulses also effect the release of gastric hormone (see NYHUS *et al.*, 1960).

Apart from the hormone gastrin, histamine too plays a role in the mechanism of acid secretion (see Section 15). Both substances specifically stimulate the secretion of acid. Initially this fact suggested gastrin and histamine might be identical, but KOMAROV (1938, 1942) amongst others demonstrated they were not. He prepared a gastrin-containing extract from the pyloric mucosa of pigs and dogs by using methods for the extraction of proteins. He then purified this extract of N-containing compounds of small molecular weight so that the preparation was free of choline and histamine. Slow intravenous injection of this extract stimulated acid secretion, but subcutaneous injection was ineffective. UVNÄS and his co-workers performed extensive experiments with gastrin extracts using KOMAROV's method of

Table 15. Secretory output following subcutaneous or intravenous administration of KOMAROV's gastrin. (r.s.s.= 2; 2 hours) (Tables XII – XVII.)

Experiment	Volume (ml.)	Total HCl (m.equiv.)	Pepsin (P.U.)	Number of expts.
conscious frogs				
control	0.359 ± 0.062	0.02193 ± 0.00474	18.6 ± 5.8	14
gastrin subcut.	0.390 ± 0.107	0.02344 ± 0.00499	19.6 ± 4.5	15
atropine subcut.	0.271 ± 0.043	0.01291 ± 0.00387	10.8 ± 4.0	15
atr.+gastr.subcut.	0.238 ± 0.035	0.01683 ± 0.00553	14.8 ± 5.1	14
animals fastened in clamps				
Ringer's sol. i.v.	0.385 ± 0.033	0.01040 ± 0.00305	9.1 ± 3.1	6
gastrin i.v.	0.294 ± 0.033	0.01179 ± 0.00257	11.4 ± 3.9	6
c.n.s. destroyed				
gastrin subcut.	0.175 ± 0.022	0.00169 ± 0.00018	7.4 ± 2.1	5
gastrin i.v.	0.171 ± 0.026	0.00169 ± 0.00043	5.0 ± 1.1	5
histamine i.v.	0.187 ± 0.037	0.00392 ± 0.00146	5.0 ± 1.0	5

preparation (UVNÄS, 1942; BAUER and UVNÄS, 1944; MUNCH-PETERSEN, RÖNNOW and UVNÄS, 1944). Only the mucosa extracts of the pyloric stomach were effective; pancreatic and salivary secretion were not stimulated; there was no effect on stomachal motility; histaminase did not change the acitivity of the preparation; the extract could be inactivated by pepsin and trypsin. These results prove that gastrin is chemically not related to histamine, but has the properties of a protein.

Several other investigators also prepared histamine-free gastrin (HARPER, 1946; JORPES *et al.*, 1952; GREGORY and TRACY, 1961). The gastrin preparation of GREGORY and TRACY, which was prepared from the pyloric mucosa of hog stomachs, was effective when administered subcutaneously, intramuscularly or, at a slow rate, intravenously. They were able to stimulate the secretion of acid, but not pepsin, in dogs and in man. GROSSMAN (1961) obtained an increase in pepsin output by using GREGORY's gastrin together with cholinergic stimulation (administration of mechothane).

As far as I am aware, gastrin preparations have been used in only two investigations on gastric secretion in *Rana*. KEETON *et al.* (1920) failed to evoke gastric secretion in *Rana esculenta* L. by administering their gastrin preparation (1915), although they succeeded with *Rana catesbyana*. CROMBACH *et al.* (1958) administered the gastrin preparation of KEETON and KOCH to *Rana esculenta* L., while secretion was evoked by distending the stomach. They only measured the peptic activity of the gastric juice. Subcutaneous injections with KEETON's gastrin failed to increase pepsin secretion.

In my experiments I initially used KOMAROV's gastrin, later I used GREGORY's gastrin, both prepared from the antral mucosa of hog stomachs. The dose of KOMAROV's gastrin used in subcutaneous injections corresponded to 20 gr. of fresh tissue per kg. bodyweight. If intravenously injected, a dose corresponding to 5 gr. of fresh tissue per kg. bodyweight was used. KOMAROV's gastrin subcutaneously administered to conscious frogs was not found to cause any increase in secretion (Table 15).

Next I tried to administer the extract by slow intravenous injection into the femoral vein. For this purpose it was necessary to fasten the animal in a clamp. A longitudinal incision was then made through the skin of the left hind leg, after which the femoral vein was exposed. Then the injection needle was inserted into the blood-vessel. As long as the needle remained in its position no loss of blood occurred. Every 10 minutes a small quantity of the extract was injected in such a way that the whole dose was administered gradually over a two hours period. Ringer's solution was used as a control. In fettered animals the secretory output was low. In the following section further data concerning the inhibitory influence of restricted mobility will be given. The point here is that no difference

in gastric secretion was found in animals injected intravenously with Ringer's solution and frogs injected with KOMAROV's gastrin. Animals in which the c.n.s. had been pithed did not show different reactions when the mucosal extract was injected subcutaneously or intravenously at a slow rate (Table 15). Neither could the secretion be stimulated by subcutaneous administration of KOMAROV's gastrin when the parasympathetic system was inhibited with atropine. This gastrin preparation appeared to be ineffective if injected subcutaneously.

In order to perform intravenous injection it was necessary to fasten the frogs, or to anaesthetize or decerebrate them. These treatments, however, have a strongly inhibiting effect on secretion. In experiments on dogs and cats it has been found that KOMAROV's gastrin is only effective if used in anaesthetized animals and only then when it is administered intravenously at a slow rate. GREGORY's gastrin on the contrary is also active in conscious dogs and men; futhermore it is very effective when subcutaneously administered. GREGORY and TRACY prepared their mucosal extract in three stages of purification which all exhibit a potent gastrin action.

Here in order to test the effect of mammalian gastrin on the gastric secretion of frogs the stages I and III are used. Stage I was administered in a dose corresponding to 75 grams of fresh tissue per kg. frog. The acid secretion was found to be somewhat stimulated (Table 16). The secretion of pepsin was uneffected. Injection of GREGORY's gastrin stage III resulted in an increased acid secretion. The pepsin output was not significantly increased.

The effect of gastrin injections on the acid secretion of the frog is represented in Fig. 10. Thus GREGORY's gastrin was found to be effective; gastrin prepared after KOMAROV failed to stimulate gastric secretion in the frog. The positive secretory reaction to mammalian gastrin suggests that in *Rana esculenta* L. a hormonal mechanism comparable to that of mammals might be involved in the regulation of gastric acid secretion.

Table 16. Secretory output following subcutaneous administration of GREGORY's gastrin. (r.s.s. = 2; 2 hours; each mean represents 15 expts.) (Tables XVIII – XXI.)

component	control	gastrin stage I	t	P
volume (ml.)	0.424 ± 0.083	0.414 ± 0.051	0.21	
free HCl (m.eq.)	0.00985 ± 0.00315	0.01511 ± 0.00325	2.09	< .05
total HCl (m.eq.)	0.01555 ± 0.00354	0.02146 ± 0.00372	2.08	< .05
buffer (m.eq.)	0.00570 ± 0.00089	0.00635 ± 0.00087	0.97	
pepsin (P.U.)	18.9 ± 6.1	15.0 ± 3.6	0.99	

component	control	gastrin stage III	t	P
volume (ml.)	0.351 ± 0.058	0.417 ± 0.040	1.82	< .05
free HCl (m.eq.)	0.01346 ± 0.00298	0.02250 ± 0.00306	4.10	< .005
total HCl (m.eq.)	0.02042 ± 0.00293	0.02996 ± 0.00347	4.07	< .005
buffer (m.eq.)	0.00696 ± 0.00128	0.00745 ± 0.00183	0.43	
pepsin (P.U.)	12.9 ± 3.7	14.1 ± 3.5	0.45	

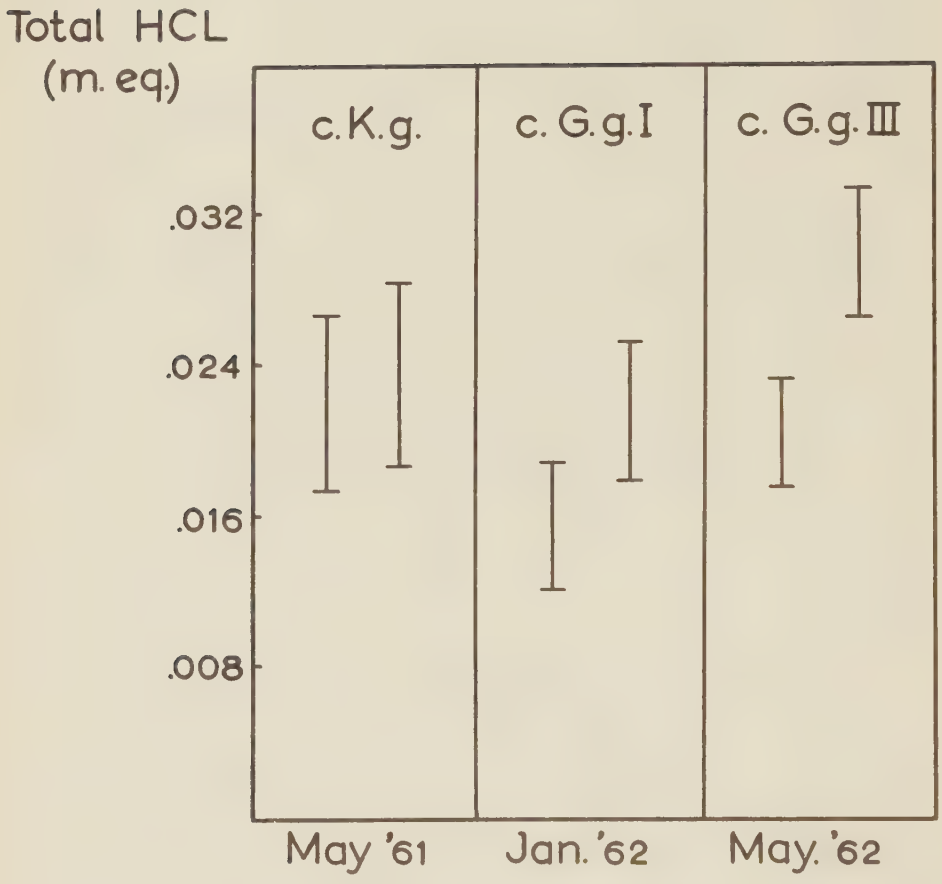

Fig.10. Total acid output in control frogs (c) and acid secretion following subcutaneous injection of KOMAROV's gastrin (K.g.) or one of two preparations of GREGORY's gastrin (G.g.). (I = 1st stage; III = 3d stage.) Data obtained from tables 15 and 16.

CHAPTER V. RELATION BETWEEN ACID AND PEPSIN SECRETION.

17. Mutual independence of acid and pepsin secretion.

In the foregoing sections several influences on acid secretion and on pepsin secretion were discussed separately. It was found that both pepsin output and acid output depend on the strength of the mechanical stimulus; administration of a larger sponge increases pepsin secretion as well as the secretion of acid. There is, however, a difference in degree: pepsin secretion reacts more strongly to an increase in mechanical stimulation than does acid secretion (see Table 17).

Table 17. Influence of strength of mechanical stimulus (r.s.s.) on acid and pepsin secretion. (Time: 4 hours; 96 gastric juice samples; figures for free HCl after KLOK and SMIT, unpubl.) (Table XXII.)

r.s.s.	free HCl in m.eq. and in per cent	pepsin in P.U. and in per cent
1	0.0081 (100%)	9.3 (100%)
3	0.0127 (157%)	30.8 (331%)

Changes in acid and pepsin secretion occurring with time do not always concur. For the two types of stimulation given in Table 18, the temporal characteristic is statistically the same for both pepsin and acid. On the other hand, this does not hold true for a single individual. Fig. 11 represents the output of acid and pepsin in three frogs. In these three arbitrarily chosen animals the acid output increases with time. The pepsin output does not follow the same course. For instance, in frog 173 the highest acid secretion coincides with a low enzyme activity.

The temporal characteristic of the secretion of a certain component is defined by the type of stimulation (Fig. 12).

Extrinsic denervation of the stomach exerts only a slight influence on gastric secretion; vagotomy does not change the acid secretion, but pepsin output is significantly diminished by vagotomy (Table 19).

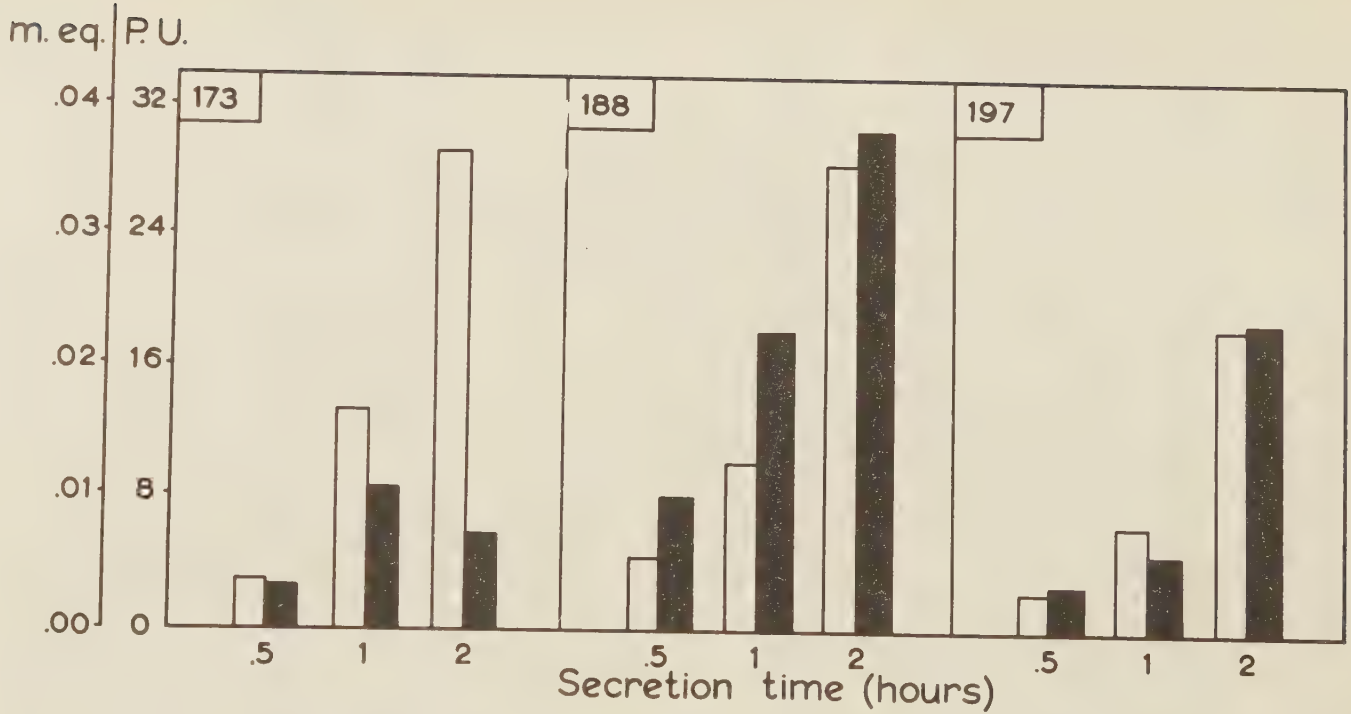

Fig.11. Total acid output in m.equiv. (open column) and pepsin output in pepsin units (solid column) of three arbitrarily chosen frogs in response to distension of the stomach in relation to secretory time. r.s.s. = 2.

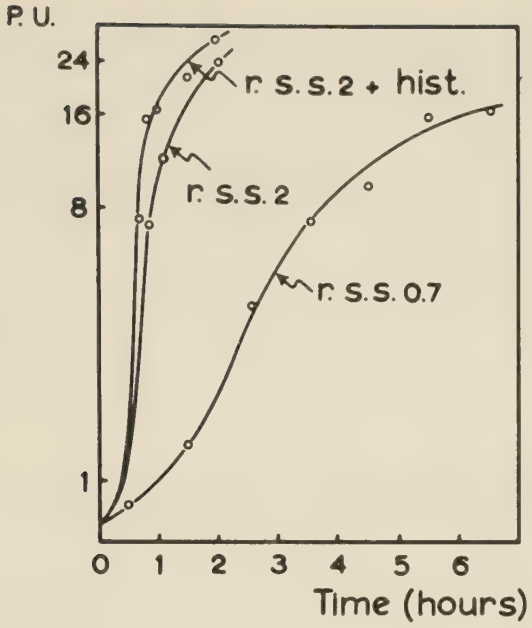

Fig.12. Temporal characteristic of pepsin secretion for several types of stimuli. Secretion evoked by a small sponge (r.s.s. = 0.7), by a large sponge (r.s.s. = 2), and by a large sponge combined with histamine injection (r.s.s. = 2 + hist.). 156 gastric juice samples.

Table 18. Absolute and relative increase of the output of acid and pepsin in relation to time. (r.s.s. = 2; 90 gastric juice samples.)

time min.	acid in m.equiv. and in per cent	pepsin in P.U. and in per cent
	without histamine	
53	0.00680 (100%)	7.0 (100%)
64	0.01006 (148%)	11.6 (166%)
120	0.02177 (320%)	24.0 (343%)
	after histamine administration	
41	0.00843 (100%)	7.0 (100%)
47	0.01421 (168%)	15.4 (220%)
58	0.01977 (234%)	16.4 (234%)
90	0.02504 (297%)	21.0 (300%)
114	0.03509 (416%)	29.0 (414%)

Table 19. Secretion before and after extrinsic denervation of the stomach. (r.s.s. = 2; 143 gastric juice samples; figures for acid after KLOK and SMIT, unpubl.) (Tables I, II and III.)

component	intact animals	coeliac nerve cut	vagotomy
free HCl in m.eq.	0.0178 ± 0.0032 (100%)	0.0188 ± 0.0027 (106%)	0.0178 ± 0.0043 (100%)
pepsin in P.U.	26.0 ± 7.3 (100%)	20.7 ± 6.1 (80%)	15.4 ± 5.6 (59%)

Table 20. Inhibiting influence of atropine on the secretion of acid and pepsin. (r.s.s. = 2; 20 mg. atropine per kg. frog injected in the ventral lymph sac.) (Tables XII, XIV, XXIII and XXIV.)

component	blank	atropine	
total HCl in m.eq.	0.02193 ± 0.00474 (100%)	0.01291 ± 0.00387 (59%)	time: 2 hours. mean body-weight: 55 g.
pepsin in P.U.	18.6 ± 5.8 (100%)	10.8 ± 4.0 (58%)	30 gastric juice samples.
free HCl in m.eq.	0.01101 ± 0.00290 (100%)	0.00637 ± 0.00194 (58%)	time: 1½ hours. mean body-weight: 51 g.
pepsin in P.U.	18.6 ± 3.2 (100%)	11.4 ± 2.4 (61%)	27 gastric juice samples.

Atropine, on the other hand, inhibits the secretion of both components approximately to the same extent (Table 20).

Vascular conditions do not influence the secretion of acid and of pepsin equally. This is manifest in animals with pithed spinal cords. Pithing causes a sharp fall of the bloodpressure and reduces acid secretion to zero, but pepsin secretion remains measurable (see Table 7 and p.34).

The strong influence of the circulation on secretion was demonstrable by measuring secretion in a number of animals, first when free, and again when clamped. When clamped, the animals probably have a high sympathico-tone which would cause, among other things, vasoconstriction in the splanchnic area. It was found that inhibition of acid secretion is stronger than that of pepsin secretion (Table 21).

Summarizing, pepsin secretion is more sensitive to vagotomy than acid secretion; on the other hand the acid secretion is more sensitive to changes of the stomachal bloodsupply. Atropine, which blocks the nerve plexus in the stomach wall, affects the secretion of both substances equally. Therefore the proportion of acid and pepsin in gastric juice depends on the nature of the factors controlling secretion.

Table 21. Influence of the immobilizing the animal on secretion. r.s.s. = 2; time: 2 hours; 24 experiments. (Tables XXV and XXVI.)

component	free animals	clamped animals	percentage reduction
volume (ml.)	0.339	0.215	37
total HCl(m.eq.)	0.01624	0.00477	71
buffer (m.eq.)	0.00647	0.00629	3
pepsin (P.U.)	15.6	8.5	46
acidity (mN)	56	18	68
pepsin conc. (P.U./ml.)	54	39	28

The possibility of interdependence between the secretion of acid and pepsin should also be considered. The function of gastric juice implies that its components might be secreted simultaneously and at approximately the same rate. On the other hand the possibility exists that under certain circumstances one component may be secreted copiously and another component sparingly, since they originate from different cells, and in *Rana* even from different glands.

A number of authors, working on gastric secretion in mammals, have investigated this problem. Their opinions fall into three categories:

1) Several authors are of the opinion that acid and pepsin secretion are not interdependent. Thus BABKIN (1929) believes in an independent regulation of the secretory activities of the several cellgroups in the gastric glands. Furthermore, the activity of the mucous cells influences the acidity of the gastric

juice by diluting and neutralizing the acid secretion. Here it must be pointed out that dilution and neutralization indeed exert an influence on the concentrations, but not on the secreted quantities. Comparison of concentrations alone may lead to erroneous conclusions, and most authors who think that interrelation does not exist base their opinion on observation of concentrations. Changes in acidity of gastric juice usually accompany changes in secretory rate (SAVICH, 1922). BUCHER and IVY (1941) investigated "histamine secretion" in dogs; they also found that the composition of the gastric juice is a function of the secretory rate. This holds true for histamine induced secretion, since the non parietal component is secreted in constant quantities whilst the parietal secretion strongly depends on the histamine doses. If secretion of both components was stimulated the relation between the composition of the gastric juice and the secretory rate disappeared. Also when there was a copious flow of acid this relation was initially found, but once the stomach was drained of neutralizing mucus by the acid secretion, the relation between acidity and secretory rate disappeared, and the acidity was kept at a high and constant level. Thus it was found that the acidity was governed to a high extent by the relative quantity of non parietal secretion (see e.g. KALK, 1932). If, for instance, the quantity of acid is correlated with the pepsin output, acidity is not necessarely related to the peptic activity of the gastric juice, since the acidity is influenced by dilution and neutralization, and the peptic activity only by dilution.

MERTEN and FETSCHER (1956) found a positive correlation in man between the quantity of free HCl and the pepsin output, but there was no correlation between the concentrations of free HCl and pepsin. In view of the absence of this correlation the authors concluded that acid and enzyme producing cells were not influenced by a common factor. However, as they did observe a good correlation between the quantities of free acid and pepsin the most plausible inference would seem to be that there did exist such a common factor. This example illustrates that comparison of concentrations can lead to incorrect conclusions.

The fact still remains that in a number of cases the composition of the gastric juice, when secreted under different circumstances, can vary considerably.

2) A second group of investigators obtained results from which it could be concluded that acid and pepsin secretion are interrelated.

HUNT (1950) found that, for human gastric secretion elicited by food or by histamine, the outputs of acid and pepsin were correlated. He contended that the parietal and the enzyme producing cells were influenced by a common factor. He suggested that this factor was the stomachal blood supply. JANOWITZ and HOLLANDER (1952) obtained from their measurements of the human basal secretion a positive correlation between pepsin and HCl output. They thought that possibly the vagus exerted a common influence on pepsin and parietal cells. OSTERBERG *et al.* (1936) and AITKEN *et al.* (1954) found a linear relationship between the concentrations of HCl and pepsin in man after injection of histamine. VILLARREAL (1953) observed a high correlation between acid and pepsin secretion of isolated mice stomachs in which secretion was evoked by means of carbachol (= carbaminoylcholine, a parasympathomimetic). HIRSCHOWITZ and LONDON (1955), investigating acid and pepsin secretion in man, found a positive correlation between these components when secretion was suppressed with atropine, C6 and Diamox, and also when it was stimulated with histamine and hypoglycaemy. HIRSCHOWITZ (1957) is of the opinion that the compound gland functions as a unit; consequently pepsin and HCl secretion would always be correlated.

3) Others, while admitting that acid and pepsin secretion may sometimes show positively correlated changes, stress that at other times secretory processes appear to be independent. OSTERBERG *et al.* (1936) point out that it is impossible to predict the acid concentration in a sample of juice from the concentration of the pepsin it contains. The only conclusion one can draw, according to these authors, is that as a rule a stomach which secretes acid in a high concentration also secretes pepsin in a high concentration. In a restricted number of cases high pepsin concentration was found in gastric juice without free HCl, or, alternatively, gastric juice samples were found with a high acidity but without measurable peptic activity. According to GLASS *et al.* (1950) the concentrations of acid, pepsin and mucoprotein in a fasting stomach can be independent of one another. Nevertheless hyperacidity is usually accompanied by hypersecretion of pepsin and mucoproteins. Finally POLINER and SPIRO (1958) also found in their patients that acid and pepsin secretion are usually, but not always, positively correlated.

18. Influence of histamine on pepsin secretion.

For some thirty years a controversy has existed over the possible influence of histamine on pepsin secretion. In 1931 VINEBERG and BABKIN found a lowering of the pepsin concentration of gastric juice when histamine was administered to dogs provided with a Heidenhain pouch. At the same time GILMAN and COWGILL (1931) obtained a similar result. They observed an initial increase of the pepsin output after administration of histamine, followed by a sharp decrease. They considered this brief rise to be the result of "washing-out" of residual enzyme from the gland lumina and folds of the mucosa. After a second histamine injection the initial rise in pepsin concentration did not recur. BOWIE and VINEBERG (1935) investigated the pepsin secretion with the aid of a histological method. A staining method considered to be specific for pepsinogen granules (devised by BOWIE, 1935), was used to determine if the number of secretory granules was lowered by the administration of histamine. The authors worked with anaesthetized dogs. After histamine injection the secretion volume increased sharply, but the number of pepsinogen granules in the cells was not significantly diminished. Stimulation of the vagus resulted in a copious flow of gastric juice, but consequently only a few granules remained in the cells. BOWIE and VINEBERG concluded that histamine stimulates only the parietal cells and not the pepsin producing cells.

BJÖRKMAN *et al.* (1943) worked with anaesthetized cats. They alternately injected histamine and stimulated the vagus. The pepsin content of the gastric juice was increased by vagal stimulation, but it did not increase after histamine injection. After a period of vagal stimulation there was a rise in the pepsin content when histamine was administered, but the authors thought that this was residual pepsin flushed from the stomach and was not actively secreted. On the other hand BUCHER and IVY (1941) using dogs provided with a Frémont pouch observed an increase in pepsin output even after a second histamine injection. To minimize the flushing effect, BUCHER, IVY and GRAY (1941) administered histamine continuously to dogs provided with vagotomized entire-stomach pouches. If the strength of the histamine infusion was increased an enlarged pepsin output followed. They came to the conclusion that pepsin secretion too was stimulated by histamine. The authors pointed out that histamine was much less effective than pilocarpine. BJÖRKMAN *et al.* (1943) thought

that these different results were possibly due to the use of conscious dogs by BUCHER and her co-workers; the occurrence of a basal pepsin secretion could not be overlooked. When histamine was administered in low concentrations, a reaccumulation of pepsin in the lumina of the glands should take place. This pepsin should be flushed after raising the concentration of histamine. This criticism was refutable because BUCHER (1949) was able to evoke pepsin secretion with histamine in anaesthetized dogs for as long as 9 hours. Vagotomy performed during these long term experiments did not inhibit the pepsin secretion.

In man also histamine elicits gastric secretion rich in acid, but poor in pepsin. Although after histamine administration the pepsin concentration decreases, the total pepsin output increases (POLLAND and BLOOMFIELD, 1931). RIVERS and VANZANT (1937) agree with the opinion that the pepsin glands are stimulated by histamine. They base their opinion on the occurrence of a temporal increase in pepsin output following a second histamine injection. JANOWITZ and HOLLANDER (1952) try to explain this phenomenon by assuming the pepsin formed during a continuous basal process is not completely flushed by the secretion elicited by the first histamine injection. BJÖRKMAN *et al.* (1943) referring to their experiments on cats point out that the pepsin content of the histamine stimulated secretion depends upon the pepsin content of the glands at the beginning of the experiment.

According to MERTEN and FETSCHER (1956) administration of histamine in man always leads to an increase in the pepsin output. Injection of a standard dose of histamine and histamine infusion results in a rise of the enzyme secretion, even when the initial value is low. The authors point out that histamine mainly stimulates the acid secretion. Pepsin secretion is stimulated to a much lesser degree; the reactions of both types of cells presenf in the gastric glands at most differ in degree.

HIRSCHOWITZ *et al.* (1957) used histamine infusions in their experiments on man. They observed that after approximately 1½ hours the increased pepsin output reached a plateau on which it could be maintained as long as the infusion of histamine was continued. FRIEDMAN *et al.* (1957) also report an active secretion of pepsin in man after repeated histamine injections.

From the above experiments one may conclude that in man pepsin secretion is stimulated by histamine. The pepsin producing cells, however, react more feebly to histamine than the parietal cells. Owing to the sharp increase in volume, which is caused by the histamine induced parietal secretion, the pepsin concentration of the gastric juice decreases. On the other hand pepsin output is increased. This increase is not merely flushing of pepsin, as is shown by the experiments with continuous histamine injection where, over long secretory periods, the pepsin output is maintained at a high level.

The same holds true for dogs. The result of one single histamine injection might suggest that the initial increase of the pepsin output is caused by flushing, but histamine infusions demonstrate that in dogs also histamine does evoke an active secretion of pepsin. HIRSCHOWITZ (1957) thinks that the increase in the pepsin content at the beginning of the secreting is probably due to a "dry" secretion of pepsinogen consisting of the zymogen of the granules, but when a copious flow of water occurs the pepsinogen is secreted in solution.

Together with active secretion, a "washing-out" probably always occurs. The smaller the active secreting is, the more pronounced the effect of flushing is likely to be.

Thus, a difference only of degree exists between the secretion of acid and pepsin when stimulated by histamine.

Rana esculenta L. is a suitable animal to use for investigation of the influence of histamine on pepsin secretion. The structure of the mammalian gastric glands, where acid and pepsin producing cells are found together, makes the existence of flushing possible. In *Rana*, however, both types of cells occur in separate glands. The peptic glands are found in the oesophagus and the HCl producing cells in the pyloric stomach (see Section 2). Because of this, a "washing-out" similar to that in mammals is not likely to occur in *Rana*. An increase of the pepsin output, therefore, can only result from active pepsin secretion.

We shall now discuss the influence of histamine on the secretion of pepsin in the same animals of which the acid secretion has been discussed in Section 15. Two series of experiments were performed: in the first, secretion is evoked by mechanical stimulation of the stomach wall (the control); in the second, the mechanically evoked secretion is further stimulated by histamine; for convenience the gastric juice obtained in this way is called "histamine juice". Three different secretory periods are used. All data of one series are arranged in groups according to gastric juice volume (see p. 59). In both series the pepsin output increases with increasing secretory time in such a way that there exists a linear relation between pepsin output and secretory period. A difference was found between the control and the histamine juice in that the histamine juice contains more pepsin than the control. When output is plotted against time, the histamine line is situated above the control line. Furthermore the slope of the histamine line is somewhat steeper than that of the control (Fig. 13 A). The increase in pepsin secretion following administration of histamine is proportionately the same as the increase in total secretory volume. Accordingly the relation between volume and pepsin activity in the histamine juice is the same as that in the control (Fig. 13 B). This means the output of pepsin during histamine stimulation is greater than in the control, but the pepsin concentration of both juices is the same (see Table 14). Since it is improbable that flushing occurs in *Rana*, it may be assumed that the increased pepsin output following histamine injection is the result of a histamine stimulated active pepsin secretion. A difference in sensitivity to histamine is found, however, between acid and pepsin secretions in that acid secretion reacts more strongly to histamine than pepsin secretion. While the pepsin concentration is not raised by histamine, the acidity increases. This difference in sensitivity is only of degree, but it has changed the composition of the gastric juice.

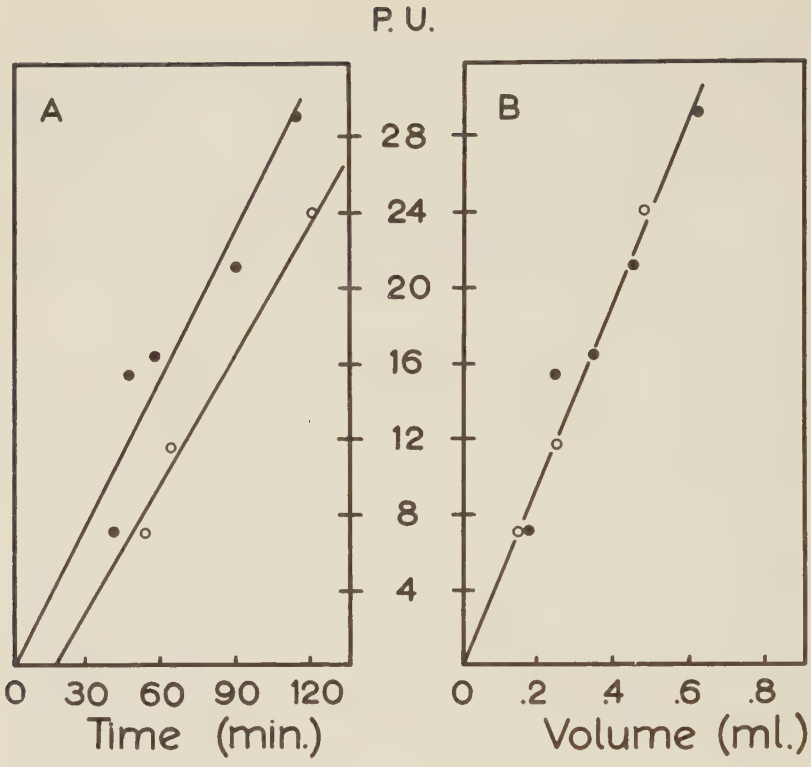

Fig.13. Relation of pepsin output in pepsin units, during mechanical stimulation of the stomach and with and without injection of histamine, to secretory time and secretory volume. Open circles : without histamine; solid circles : with histamine; r.s.s. = 2; 90 gastric juice samples.

The experiments described show that *pepsin output is increased by histamine. This increase is caused by intensified active secretion of pepsin.*

19. Relation between acid and pepsin secretions during mechanical stimulation and after histamine administration.

The experiments discussed in Section 15 may also be used to compare the outputs of the several components of the gastric juice. We are now especially concerned with a possible correlation between pepsin and acid output. If we plot the mean output figures of acid against those of pepsin a linear relationship appears (Fig. 14 A).

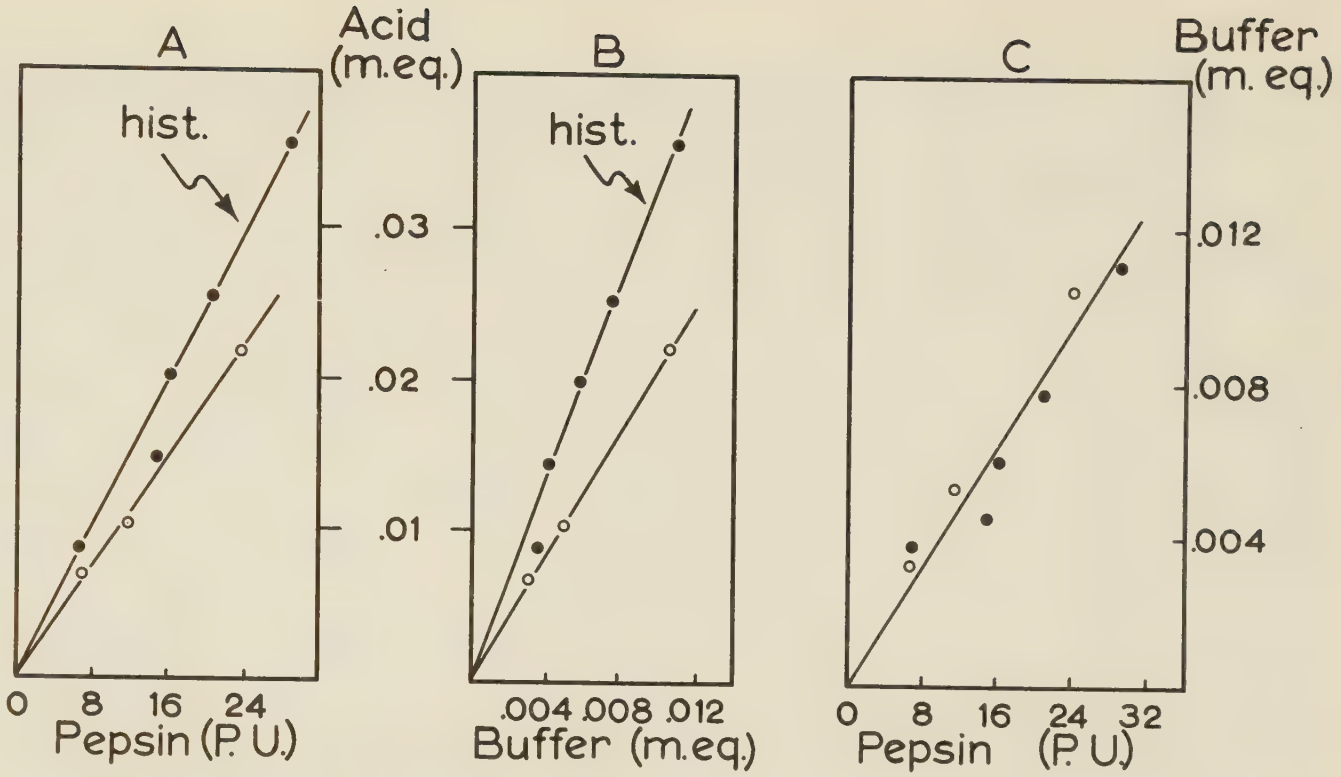

Fig. 14. Relation between several components of the gastric juice. Secretion evoked by mechanical stimulation of the stomach, with and without histamine administration. Open circles : without histamine; solid circles : with histamine; r.s.s. = 2; statistical curves for 90 samples of gastric juice.

Furthermore, positive correlations between acid and buffer, and buffer and pepsin secretions are found. For the "histamine secretion" the relation between components is different from that of the "control secretion". The slope of the histamine line is steeper, i.e. in the histamine induced secretion the increase in the acid output is greater than that of the pepsin and buffer secretions. Although histamine stimulates acid secretion more strongly than pepsin secretion (cf. Section 18), a correlation between acid and pepsin outputs still remains after histamine injection. It must be borne in mind that the points in the graphs of Fig. 14 are averages; the separate values may deviate considerably from the straight line relationship.

It is not surprising that a relationship between acid and pepsin secretion was found, since care was taken to apply stimuli of equal strength to all animals within a series. Under these conditions the correlation is retained throughout the time the stimulus is acting; application of another type of stimulus may result in a different relation between acid and pepsin outputs. If one type of stimulus is replaced by another, a transitional stage would take place prior to the establishment of a new relationship. Likewise, deviation from the usual relation between acid and pepsin occurring with a given type of stimulus will appear at the beginning and at the end of a secretory period. This deviation occurs because of a difference in sensitivity of acid and pepsin producing cells to the stimulus, and it also depends on the duration of stimulation. The latter fact relates to the specific temporal course of the secretion of the separate components. The manner in which acid and pepsin secretion both react to mechanical stimulation of the stomach wall has already been discussed in Section 17. They do not react in the same manner to alterations in the strength of the stimulus; the temporal characteristic of a given stimulus is not always the same for acid and pepsin secretion; extrinsic denervation of the stomach has a greater influence on pepsin secretion than on the secretion of acid; on the other hand, acid secretion is more sensitive to lowering of the bloodpressure. The secretory mechanisms of the components are sensitive to the same influences, but to a different extent. It follows that the regulation of the acid and pepsin secretions are always coupled with each other in one way or another: the contents of the stomach exert their influence on both, both are influenced by alterations in the bloodpressure, and variations of the sympathetic tone exert their influence on the secretion of both components. Thus if the circumstances are relatively constant, correlation between the secretion of acid and pepsin must result. This does not mean, however, that the parallelism between secretion of the components is inflexible. The type of stimulus defines the relative quantity of each.

DISCUSSION

In *Rana* secretion of gastric juice can be evoked by mechanical stimulation of the stomach wall. The quantity of the secretion is, within certain limits, related to the strength of the mechanical stimulus. Long reflex paths are not used, since mechanical stimuli remain effective after complete extrinsic denervation of the stomach, although secretion of pepsin is somewhat diminished by vagotomy. Apparently the neurogenic regulatory mechanism is primarily located in the stomach wall. Because secretion can be blocked by atropine, it must be controlled by a local cholinergic reflex.

This mechanism may be compared with that found in mammals. LIM *et al.* (1925) were able to evoke gastric secretion in dogs provided with entire-stomach pouches by inflating a balloon in the pouch. The pouch responded to this stimulation even after complete denervation, but secretion was completely abolished by atropine.

GROSSMAN, ROBERTSON and IVY (1948) were able to evoke secretion in a subcutaneously transplanted, and thus completely denervated, fundic pouch by distension of the main pouch. This proved the existence of a hormonal secretory mechanism. Procainization of the main pouch abolished the secretory response in the transplanted pouch. Similar results were obtained with atropine. Thus the nervous mechanism that involves the release of the secretory hormone reacts to mechanical stimuli. The receptive part of this mechanism can be blocked by procaine and the efferent part by atropine (GROSSMAN, 1950).

HUNT and MACDONALD (1952) experimenting with man gave test-meals of pectin. The properties of pectin are such that no cephalic phase of secretion occurs; it stimulates secretion only by distending the stomach. In the experiments of HUNT and MACDONALD the secretory output increased with increasing volume of the test-meal. When, however, quantities beyond a particular limit were eaten, inhibition of secretion took place. Similarly SCHOFIELD (1959) found that, in dogs provided with a denervated pouch, meals of a large volume inhibited pepsin secretion. This experiment showed inhibition was independent of the extrinsic innervation. Inhibition of pepsin secretion is correlated with suppression of the motility of the stomach. Both processes are suppressed by atropine and hexamethonium, and it is suggested that both depend on the same activity of the intramural nerve plexuses.

WOODWARD and DRAGSTEDT (1960) working with dogs provided with an antrum pouch and a Heidenhain pouch found that mechanical stimulation of the antrum pouch by means of a balloon evoked secretion in the Heidenhain pouch and increased the antral motility. Anticholinergic drugs blocked this increase of motility, and at the same time the release of gastrin was prevented. From this the importance of the intramural antral nerves in the control of the endocrine function of the antrum becomes evident.

In *Rana* the extrinsic nerves mainly influence motility and tone of the stomach (see Section 3). Unfortunately the results of the investigations of this activity are not in complete agreement. In general the vagus has a stimulatory effect on stomachal motility, but the sympathetic influence seems to be not always inhibitory. The antagonism between parasympathetic and sympathetic systems in their control of stomachal motility is apparently not very marked.

It is striking that vagotomy reduces somewhat the secretion of pepsin, but not that of acid. If a decreased motility of the stomach in vagotomized frogs causes reduced pepsin secretion, why is the output of acid not also diminished? Results (Table 17) show that pepsin secretion reacts more strongly to an increase in strength of the mechanical stimulus than acid secretion. Consequently it is possible that lessened mechanical stimulation caused by diminished motility has a proportionately greater influence on pepsin secretion than on the secretion of acid. This explanation is tentative. It seems improbable that the decrease in pepsin secretion following vagotomy is caused by alterations of the bloodflow, since acid secretion was more sensitive to circulatory variations than secretion of pepsin (Table 21). If changes in the bloodflow occur, primarily acid secretion will be influenced. Another possible explanation might lie in a cephalic phase, which acts in intact frogs and is abolished by vagotomy. It is true that WOLVEKAMP and TINBERGEN (1942) were not able to demonstrate the existence of a cephalic phase in *Rana esculenta* L., but the gravimetric method they used to determine the peptic activity of gastric juice was not likely to permit accurate measurement. Since vagotomy inhibits the secretion of pepsin the possibility of the occurrence of a cephalic phase in *Rana* should still be accepted.

The following arguments provide strong evidence that the blood supply of the stomach greatly influences secretion:

a) Destruction of the c.n.s. weakens the circulation and inhibits the secretion. This inhibition is not caused by the interruption of a reflex path, because extrinsic denervation of the stomach does not inhibit secretion.

b) Nembutal lowers the bloodpressure and inhibits secretion.

c) In frogs which are continually disturbed, secretion is decreased. It is suggested that the increased adreno-sympathetic tone has a vasoconstrictory effect in the splanchnic area. Again a positive correlation is found between limited bloodflow and decreased secretion.

d) In frogs which are fastened in a clamp the secretory response to stomachal stimulation is markedly reduced. The same reasoning holds as in c).

e) Sodium nitrite has a vasodilatory action. To obtain vasodilation in the splanchnic area, however, the stomachal branch of the splanchnic nerve must be cut. Administration of sodium nitrite then gives a substantial rise in secretion (Table 10).

As in mammals, gastrin and histamine preparations exert a distinct stimulatory effect on acid secretion. However, in the frog natural occurrence of neither histamine nor gastrin has been proved.

The composition of the gastric juice depends upon the secretory rate of the separate components. These secretory rates are determined by the strength and type of stimulus. Total output depends on the duration of the secretion time, but there is no strict relationship between duration of the stimulus and concentrations of the components. These concentrations also depend on the strength and type of stimulus.

It is not possible to calculate from my data the primary acidity of the HCl secretion. For this purpose it is necessary to measure the total chloride content of the gastric juice, and also to estimate the diluting and neutralizing effects of the non-acid secretion.

The peptic activity of each gastric juice sample was measured at a standard pH, and this activity was used as a measure for the quantity of pepsin in these samples. The proteolytic activity in the stomach depends on the pH of the gastric contents, and the pH again is influenced by the composition of the food.

It is not only the volumes of acid and pepsin secreted which are of biological significance, but also the cooperative activity between them. In the chyme, a gastric juice containing much acid and little pepsin may have a high digestive ability, because the pH is favourable for the enzymatic activity. On the other hand, the unfavourable pH of a gastric juice containing a small quantity of acid and much pepsin may be offset by the large quantity of pepsin. In animals which secrete small quantities of acid, much pepsin can be found, and *vice versa* (MENNEGA, 1938; VONK, 1939). VONK points out that one does not obtain a correct impression of enzymatic activity in vivo by experiments in vitro, since enzyme activity in vivo is strongly influenced by stratification of the chyme and by gastric movements. Strict adjustment of the pH of the gastric contents to the pH-optimum of peptic activity is probably less important than sometimes is suggested. Peptic activity is still considerable at one pH unit above and below the pH-optimum of the enzyme.

That the secretory rate of the several components depends on the distension of the stomach, is also an important fact. Emptying of the stomach depends on its motility, which in turn is under the influence of the extrinsic innervation; the innervation therefore in-

fluences secretion indirectly. Further, after an animal has fed, secretagogues cause a hormonal stimulation of acid secretion.

Accordingly there are many factors influencing peptic activity in the chyme of which we do not know the quantitative effects. Close correlation between acid and pepsin secretion is not as biologically important as it is sometimes considered to be. In *Rana* this correlation is statistically significant, but pepsin output in a given specimen cannot be predicted on the basis of its acid secretion.

Comparison of the regulation of gastric secretion between classes of vertebrates is difficult.

Little is known about gastric secretion in fishes; only Elasmobranchs have been investigated, and unfortunately the results are not consistant. In sharks there is apparently some relation between secretion and digestion of food. SULLIVAN (1905) observed that the highest acidity in sharks occurs when the stomach is filled with partly digested food. The contents of a fasting stomach have a practically neutral pH. DOBREFF (1927) and BABKIN *et al.* (1935 b) on the contrary found a continuous acid secretion in Elasmobranchs. In *Scylliorhinus* DOBREFF was unable to influence secretion by means of pilocarpine, acetyl choline, histamine and atropine. Furthermore he did not succeed in demonstrating the existence of a "psychic" secretion. He supposed that the regulation of gastric secretion had a hormonal origin. BABKIN *et al.* failed to evoke secretion in rays by distension of the stomach, or stimulation of the splanchnic nerves, or by administration of adrenaline and histamine. On the other hand they could elicit a "paralytic" secretion by destruction of the spinal cord. Since adrenaline, a vasoconstrictor, prevented this secretion, they thought vasodilation would stimulate it. They assumed that secretion in Elasmobranchs is under vascular or hormonal control. Neither DOBREFF nor BABKIN *et al.* were able to demonstrate any nervous influence on gastric secretion in their experimental animals. This is the more striking since the motor innervation of the stomach is well developed (BABKIN *et al.*, 1934, 1935 a). UNGAR (1935) was the only investigator who, working with perfused isolated stomachs of *Squalus*, *Scylliorhinus* and *Torpedo*, obtained some evidence that histamine and acetyl choline stimulated secretion.

Certainly in *Rana* there is a nervous regulation. This is mediated by the parasympathetic system, and mainly by the intramural gastric plexus. Vascular reactions, and probably hormones, also control secretion. The results of experiments on fishes and amphibians are too few and too contradictory to permit comparison of their regulatory mechanisms.

In mammals hormonal regulation is important; vascular influences are less conspicuous. Nervous control is excercised by the vagus. This neurogenic regulatory mechanism is the more important as it starts the whole train of secretory and digestive processes in the stomach. Recent investigations by THEIN and SCHOFIELD (1959) and WOODWARD and DRAGSTEDT (1960) seem to prove that this nervous control is mediated by release of gastrin.

Some anatomical and functional aspects of gastric innervation should be briefly discussed.

Elasmobranchs possess a well-developed autonomic system containing parasympathetic and sympathetic elements. The abdominal organs are sympathetically innervated; the parasympathetic innervation is almost entirely confined to the organs in the head (YOUNG, 1933). The stomach is the only abdominal organ which possesses a double autonomic innervation. In Elasmobranchs, however, the functional antagonism between the sympathetic and parasympathetic systems found in mammals appears to be absent; vagal stimulation causes stomachal motility, but so does splanchnic stimulation; both systems seem to act synergistically.

In the digestive tract of teleosts too, the stomach is the only organ possessing a double innervation. Both the vagus and the sympathetic system send motor fibres to the stomach. It appears from the experiments of YOUNG (1936) that pharmacologically and physiologically the visceral motor nerves cannot easily be divided into sympathetic and parasympathetic systems. No antagonism between vagal and sympathetic fibres has been found.

In amphibians the sacral part of the parasympathetic system appears for the first time; the number of doubly innervated organs has obviously increased. Still a distinct antagonism between vagus and splanchnic activity in the stomach does not occur.

In mammals the majority of the organs possess a double autonomic innervation in which a marked antagonism can be observed, although excitatory and inhibitory properties do not belong to particular systems. In some organs the sympathetic is inhibitory and the parasympathetic excitatory; in others the reverse is true. In the digestive tract the parasympathetic system exerts an excitatory influence on motility, while the sympathetic system and adrenaline have an inhibitory effect. BABKIN (1946) points out that the gastric glands do not possess a well-defined antagonistic automatic innervation, since the sympathetic net does not appear to supply secretory fibres to the gastric glands.

Within the vertebrates there is an evolutionary trend towards

clearer delimitation of the control excercised by the two sub-divisions of the autonomic nervous system. When a comparison of the motor effects among the different classes of vertebrates is made, one sees that the antagonism between the sub-divisions becomes more pronounced in the higher systematic groups. This increasing antagonism, however, does not hold for the regulation of gastric secretion. Initially this regulation is hormonal, but a nervous regulatory mechanism in which only the parasympathetic part of the autonomic system is concerned, becomes more and more predominant.

SUMMARY.

1) In *Rana esculenta* L. secretion of gastric juice was evoked by mechanical stimulation of the stomach.

2) The output of gastric juice was proportional to the strength and the duration of the mechanical stimulus.

3) "Spontaneous" secretion was found to be negligible compared with mechanically induced secretion.

4) Frogs in which the stomachal branch of the splanchnic nerve (i.e. the sympathetic stomachal supply) was cut showed a normal secretory response to the mechanical stimulus.

5) Double vagotomy did not result in the abolishing of the secretory response to mechanical stimulation of the stomach, although the pepsin secretion was reduced by vagotomy to approximately 60 per cent of the original.

6) No secretion via a reflex in the sympathetic system could be demonstrated in *Rana*. An indirect sympathetic influence, however, did exist, for sympathetically induced vasoconstriction led to a lowering of the secretory output.

7) Destruction of the central nervous system resulted in a strong reduction in the secretory output. This reduction seemed to be caused by the greatly reduced blood supply which results from destruction of the c.n.s.

8) Administration of the sympathicolytic ergotamine did not change the secretory response significantly. Hexamethonium chloride too did not bring about a definite change. Administration of the parasympathicolytic atropine sulphate led to an inhibition of secretion; this occured in intact frogs and frogs in which the stomach was partly or totally deprived of its extrinsic nerve supply. The secretory response to the mechanical stimulus was under the control of cholinergic fibres of the intramural nerve plexus.

9) The rate of bloodflow of the oesophagus and the stomach influenced the secretion of gastric juice. Vasoconstriction in the splanchnic area led to a lowering of the secretory output. In frogs which were kept at a high level of activity, the secretion was strongly inhibited, this probably being due to a high adreno-sympathetic tone.

10) Subcutaneous administration of histamine caused a marked increase in the HCl output. Pepsin output was also increased by histamine, although only to a small degree. This increase was not caused by flushing of preformed enzyme, but by active secretion of pepsin.

11) A subcutaneous injection of a gastrin preparation extracted from the antral mucosa of hogs resulted in an increase of the acid secretion in conscious frogs. This was true only for GREGORY's gastrin preparation; administration of KOMAROV's preparation produced no significant increase in the secretion.

12) Variations in the quantities of acid and pepsin secretions do not always run parallel. The composition of the gastric juice depends upon the regulatory influences which are acting during secretion. There is strong evidence that secreting of acid and of pepsin are partly independent processes.

13) It is concluded that in *Rana esculenta* L. secreting of gastric juice is regulated by neurogenic, vascular and hormonal mechanisms.

14) The nerve fibres of the autonomic system, which regulate gastric secretion in *Rana*, are apparently cholinergic. This corresponds to the situation in mammals. In *Rana*, as in Elasmobranchs, there is an indirect sympathetic influence on secretion, which is mediated through vasomotor reactions. A hormonal secretory mechanism seems likely to occur throughout the vertebrate group.

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Table III. Pepsin output in P.U. of frogs after double vagotomy combined with severing of the coeliac nerve. Secretion evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration: 2 hours). Subcutaneous injection with Ringer's solution, di-hydroergotamine (DHE), hexamethoniumchloride (C6), and atropine sulphate. (mean bodyweight: 64.4 grams; 77 experiments.)

Ringer 28 ml/kg.	DHE 4 mg/kg.	C6 45 mg/kg.	Atropine 20 mg/kg.
39.4	35.6	10.0	4.7
49.5	10.2	9.9	11.1
7.0	9.0	18.9	6.9
21.2	13.4	12.1	7.1
5.5	26.9	14.3	12.5
13.1	6.4	2.9	17.2
16.6	34.3	7.5	15.4
17.3	22.6	13.3	19.0
4.6	9.9	1.1	6.4
12.8	0.5	2.4	12.5
18.0	16.8	3.0	10.4
21.4	4.2	8.2	9.5
11.6	17.2	10.6	12.0
11.3	18.0	1.3	5.7
8.2	13.5	5.8	6.8
6.0	22.8	2.9	9.5
15.2	13.3	2.7	5.9
12.2	17.1	3.0	10.6
11.7	20.2	3.4	
6.3	12.7		
15.4	16.2	7.0	10.2

Table IV. Acid secretion (m.equiv."free HCl") of intact frogs in which secretion has been evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration: 2 hours). Subcutaneous injection with Ringer's solution, atropine sulphate, and a combination of atropine sulphate and histamine. (mean bodyweight: 55.2 grams; 60 experiments.)

Ringer 28 ml/kg.	Atropine 20 mg/kg.	Atropine 20 mg/kg. +histamine 0.5 mg/kg.
0.01819	0.00544	0.03086
0.00901	0.00000	0.04127
0.02211	0.00767	0.02617
0.00810	0.00121	0.03666
0.01655	0.00648	0.04086
0.03130	0.00203	0.02422
0.00000	0.00443	0.05397
0.00580	0.00470	0.01593
0.00835	0.00885	0.02227
0.00806	0.00987	0.03917
0.01529	0.00633	0.04158
0.02680	0.00336	0.03884
0.00715	0.00513	0.00448
0.01574	0.01019	0.03209
0.01922	0.01158	0.04160
0.00921	0.00475	0.02282
0.02299	0.00279	0.04489
0.01341	0.00694	0.00954
0.01226	0.00000	0.03478
0.02673	0.00837	0.03477
0.01481	0.00551	0.03184

Table V. Gastric secretion of intact frogs. Secretion elicited by distension of the stomach (r.s.s.= 2; duration: 30 minutes). Mean bodyweight: 55.5 grams.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (P.U./ml.)
173	0.170	0	0.00371	0.00371	2.8	0	22	22	16
174	0.155	0	0.00221	0.00221	3.0	0	14	14	20
175	0.139	0.00058	0.00273	0.00215	3.6	4	20	15	26
186	0.112	0.00061	0.00264	0.00203	5.2	5	24	18	46
188	0.208	0.00241	0.00563	0.00322	8.2	12	27	15	40
193	0.173	0	0.00238	0.00238	2.6	0	14	14	16
197	0.124	0.00004	0.00307	0.00303	3.0	0	25	24	24
199	0.168	0	0.00150	0.00150	2.8	0	9	9	16
201	0.170	0	0.00161	0.00161	4.6	0	9	9	26
202	0.153	0.00096	0.00285	0.00189	2.6	6	19	12	18
203	0.213	0.00102	0.00532	0.00430	9.4	5	25	20	44
204	0.136	0	0.00220	0.00220	4.4	0	16	16	32
205	0.240	0.00050	0.00504	0.00454	6.8	2	21	19	28
206	0.146	0	0.00347	0.00347	6.8	0	24	24	46
207	0.282	0	0.00276	0.00276	6.2	0	10	10	22
mean	0.173	0.00041	0.00314	0.00273	4.8	2	19	16	28

Table VI. Gastric secretory output of intact frogs. Secretion evoked by mechanical stimulation of the stomach combined with subcutaneous injection of histamine (r.s.s.= 2; duration: 30 minutes; 0,5 mg histamine-diHCl per kg bodyweight). Mean bodyweight: 55.5 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
173	0.391	0.01574	0.02212	0.00638	5.6	40	57	16	14
174	0.193	0.00378	0.00718	0.00340	6.8	20	37	18	36
175	0.250	0.00549	0.00962	0.00413	4.2	22	38	17	16
186	0.153	0.00126	0.00432	0.00306	7.0	8	28	20	46
188	0.311	0.00854	0.01370	0.00516	11.0	27	44	17	36
193	0.158	0.00175	0.00558	0.00383	5.8	11	35	24	36
197	0.190	0.00495	0.00800	0.00305	4.8	26	42	16	26
199	0.193	0	0.00300	0.00300	5.8	0	16	16	30
201	0.196	0.00704	0.01167	0.00463	6.8	36	60	24	34
202	0.129	0.00118	0.00474	0.00356	6.2	9	37	28	48
203	0.378	0.00935	0.01824	0.00889	6.2	25	48	24	16
204	0.261	0.00203	0.00758	0.00555	4.6	8	29	21	18
205	0.302	0.00444	0.00949	0.00505	5.2	15	31	17	18
206	0.267	0.00181	0.00644	0.00463	5.6	7	24	17	20
207	0.341	0.00200	0.00719	0.00519	8.0	6	21	15	24
mean	0.248	0.00462	0.00926	0.00464	6.2	17	36	19	28

Table VII. Gastric secretory output of intact frogs. Secretion evoked by distension of the stomach (r.s.s.= 2; duration: 1 hour). Mean bodyweight: 56.1 grams.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
172	0.235	0.00912	0.01500	0.00588	11.8	39	64	25	50
173	0.280	0.01030	0.01665	0.00635	8.6	37	59	23	30
174	0.131	0.00444	0.00758	0.00314	3.0	34	58	24	22
175	0.184	0.01008	0.01300	0.00292	4.8	55	71	16	26
183	0.298	0.00588	0.01172	0.00584	10.0	20	39	19	34
186	0.110	0.00421	0.00791	0.00370	22.4	38	72	34	204
188	0.210	0.00783	0.01279	0.00496	18.4	37	61	24	88
189	0.248	0.00132	0.00408	0.00276	8.4	5	16	11	34
190	0.111	0	0.00294	0.00294	5.2	0	26	26	46
191	0.291	0	0.00695	0.00695	13.8	0	24	24	48
193	0.239	0.00401	0.00764	0.00363	8.4	17	32	15	36
195	0.169	0.00571	0.00865	0.00294	6.6	34	51	17	40
196	0.243	0.00038	0.00458	0.00420	7.0	2	19	17	28
197	0.178	0.00523	0.00840	0.00317	4.8	29	47	18	26
198	0.235	0	0.00342	0.00342	9.0	0	15	15	38
mean	0.209	0.00424	0.00831	0.00407	9.4	22	42	20	50

Table VIII. Gastric secretory output of intact frogs. Secretion evoked by mechanical stimulation of the stomach and stimulated by a subcataneous injection of histamine (r.s.s.= 2; duration: 1 hour; 0.5 mg histamine-diHCl per kg). Mean bodyweight: 53 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
173	0.393	0.03223	0.03868	0.00645	28.6	82	98	16	74
174	0.228	0.01672	0.02142	0.00470	20.4	73	94	21	88
175	0.260	0.02000	0.02378	0.00378	12.8	77	91	14	50
183	0.412	0.03047	0.03689	0.00642	36.0	74	90	16	88
186	0.203	0.01116	0.01577	0.00461	33.2	55	78	23	162
188	0.314	0.02082	0.02711	0.00629	17.8	66	86	20	56
189	0.288	0.01109	0.01484	0.00375	27.2	38	52	14	94
190	0.361	0.02047	0.02618	0.00571	20.6	57	73	16	56
191	0.524	0.02424	0.03514	0.01090	20.0	46	67	21	38
193	0.367	0.00626	0.01154	0.00528	12.8	17	31	14	34
195	0.321	0.01069	0.01528	0.00459	20.8	33	48	15	64
196	0.407	0.01084	0.01678	0.00594	12.0	27	41	14	30
197	0.476	0.01038	0.01517	0.00479	19.8	22	32	10	40
198	0.376	0.00620	0.00989	0.00369	11.2	16	26	10	30
mean	0.352	0.01654	0.02203	0.00549	20.8	49	65	16	64

Table IX. Gastric secretory output of intact frogs in which secretion has been evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration 2 hours). Mean bodyweight: 55.5 grams.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
173	0.446	0.02381	0.03650	0.01269	5.8	53	82	28	14
174	0.108	0.01155	0.01614	0.00459	10.8	107	149	43	100
175	0.190	0.01724	0.02352	0.00628	7.6	91	124	33	40
186	0.171	0.01198	0.01957	0.00759	34.0	70	114	44	198
188	0.251	0.02548	0.03567	0.01019	30.6	102	142	41	122
193	0.250	0.01037	0.01810	0.00773	21.2	41	72	31	84
197	0.318	0.01617	0.02349	0.00732	19.2	51	74	23	60
199	0.378	0.00216	0.00918	0.00702	23.8	6	24	19	62
201	0.458	0	0.00840	0.00840	14.8	0	18	18	32
202	0.377	0.01537	0.02750	0.01213	24.2	41	73	32	64
203	0.595	0.01205	0.02358	0.01153	38.0	20	40	19	64
204	0.287	0	0.00567	0.00567	7.8	0	20	20	28
205	0.617	0.01117	0.02256	0.01139	24.6	18	37	18	40
206	0.445	0.01028	0.01805	0.00777	16.6	23	41	17	38
207	0.715	0.01086	0.02671	0.01585	48.2	15	37	22	68
mean	0.374	0.01190	0.02098	0.00908	21.8	43	70	27	68

Table X. Gastric secretion during mechanical stimulation of the stomach following subcutaneous injection of histamine (r.s.s.= 2; duration: 2 hours; 0.5 mg histamine-diHCl per kg). Mean bodyweight: 55.5 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
173	0.586	0.06550	0.07655	0.01105	26.0	112	131	19	44
174	0.320	0.02052	0.02749	0.00697	23.2	64	86	22	72
175	0.447	0.02874	0.03591	0.00717	9.4	64	80	16	22
186	0.326	0.02269	0.03012	0.00743	42.6	70	92	23	130
188	0.574	0.03667	0.04650	0.00983	58.8	64	81	17	102
193	0.635	0.01680	0.02680	0.01000	29.0	26	42	16	46
197	0.173	0.01784	0.02293	0.00509	12.0	103	133	30	70
199	0.504	0.00846	0.01705	0.00859	17.8	17	34	17	36
201	0.495	0.00371	0.01410	0.01039	23.6	7	28	21	48
202	0.657	0.01736	0.02786	0.01050	27.2	26	42	16	42
203	0.613	0.02129	0.03308	0.01179	21.0	35	54	19	34
204	0.475	0.02063	0.03138	0.01075	25.6	43	66	23	54
205	0.747	0.02124	0.03482	0.01358	29.0	28	47	18	38
206	0.632	0.01550	0.02460	0.00910	29.8	25	39	14	48
207	0.769	0.01368	0.02847	0.01479	29.0	17	36	19	36
mean	0.532	0.02204	0.03184	0.00980	27.2	47	66	19	54

Table XI. Gastric acid output (m.equiv.) of intact frogs with and without subcutaneous injection of histamine at various secretory times. Secretion elicited by mechanical stimulation of the stomach (r.s.s. = 2). Mean body-weight : 55.2 g.

Number	no injection			histamine		
	½ hour	1 hour	2 hours	½ hour	1 hour	2 hours
172		0.01500				
173	0.00371	0.01665	0.03650	0.02212	0.03868	0.07655
174	0.00221	0.00758	0.01614	0.00718	0.02142	0.02749
175	0.00273	0.01300	0.02352	0.00962	0.02378	0.03591
183		0.01172			0.03689	
186	0.00264	0.00791	0.01957	0.00432	0.01577	0.03012
188	0.00563	0.01279	0.03567	0.01370	0.02711	0.04650
189		0.00408			0.01484	
190		0.00294			0.02618	
191		0.00695			0.03514	
193	0.00238	0.00764	0.01810	0.00558	0.01154	0.02680
195		0.00865			0.01528	
196		0.00458			0.01678	
197	0.00307	0.00840	0.02349	0.00800	0.01517	0.02293
198		0.00342			0.00989	
199	0.00150		0.00918	0.00300		0.01705
201	0.00161		0.00840	0.01167		0.01410
202	0.00285		0.02750	0.00474		0.02786
203	0.00532		0.02358	0.01824		0.03308
204	0.00220		0.00567	0.00758		0.03138
205	0.00504		0.02256	0.00949		0.03482
206	0.00347		0.01805	0.00644		0.02460
207	0.00276		0.02671	0.00719		0.02847
mean	0.01096			0.02102		

Table XII. Secretory output in response to mechanical stimulation of the stomach. (r.s.s.= 2; duration: 2 hours). Mean bodyweight: 55 grams.

Number	volume (ml.)	total HCl (m.eq.)	pepsin (P.U.)	acidity (mN)
173	0.569	0.04830	19.2	85
174	0.168	0.01184	13.0	70
175	0.261	0.02136	10.4	82
186	0.223	0.01249	38.2	56
188	0.420	0.02044	51.2	49
193	0.428	0.01159	24.0	27
197	0.209	0.01668	7.4	80
199	0.488	0.02694	13.0	55
201	0.286	0.02629	12.0	92
202	0.347	0.02908	12.6	84
203	0.294	0.02705	11.4	92
204	0.439	0.01976	14.0	45
205	0.359	0.02043	14.0	57
207	0.530	0.01480	20.8	28
mean	0.359	0.02193	18.6	64

Table XIII. Secretory output in response to mechanical stimulation of the stomach following subcutaneous injection of KOMAROV's gastrin (r.s.s.= 2; duration: 2 hours; gastrin preparation corresponding to 20 grams of fresh tissue per kg. bodyweight).

Number	volume (ml.)	total HCl (m.eq.)	pepsin (P.U.)	acidity (mN)
173	0.492	0.03886	24.0	79
174	0.188	0.01162	11.6	62
175	0.340	0.02340	10.4	69
186	0.140	0.01024	26.4	73
188	0.529	0.02585	35.4	49
193	0.486	0.02199	23.6	45
197	0.270	0.03135	9.8	116
199	0.459	0.03056	16.0	67
201	0.267	0.00680	17.0	25
202	0.403	0.01864	27.6	46
203	0.310	0.02599	17.0	84
204	0.171	0.00851	11.8	50
205	0.372	0.03274	17.8	88
206	0.396	0.03696	9.6	93
207	1.031	0.02805	37.4	27
mean	0.390	0.02344	19.6	65

Table XIV. Secretory output in response to mechanical stimulation of the stomach following subcutaneous injection of atropine (r.s.s.= 2; duration: 2 hours; 20 mg. atropine-sulphate per kg.).

Number	volume (ml.)	total HCl (m.eq.)	pepsin (P.U.)	acidity (mN)
173	0.265	0.03255	6.8	123
174	0.184	0.02224	7.0	121
175	0.219	0.01842	7.8	84
186	0.158	0.00835	14.6	53
188	0.461	0.01997	39.2	43
193	0.309	0.01272	6.4	41
197	0.234	0.01279	5.6	55
199	0.238	0.00233	9.4	10
201	0.259	0.00828	10.4	32
202	0.226	0.00351	7.0	16
203	0.183	0.00454	6.0	25
204	0.270	0.01220	9.0	45
205	0.248	0.00806	9.4	33
206	0.394	0.01584	12.4	40
207	0.419	0.01185	11.8	28
mean	0.271	0.01291	10.8	50

Table XV. Secretory output in response to mechanical stimulation of the stomach following a combined subcutaneous injection of atropine and KOMAROV's gastrin (r.s.s.= 2; duration: 2 hours; 20 mg. atropine sulphate per kg + antral extract corresponding to 20 grams of fresh tissue per kg.). Mean bodyweight: 55 grams.

Number	volume (ml.)	total HCl (m.eq.)	pepsin (P.U.)	acidity (mN)
173	0.352	0.04950	14.0	141
174	0.211	0.01477	5.0	70
175	0.227	0.01371	5.4	60
186	0.135	0.00603	17.6	45
188	0.348	0.01740	31.2	50
193	0.252	0.00906	5.0	36
197	0.231	0.02315	6.2	100
199	0.179	0.00758	7.4	42
201	0.267	0.01357	5.8	51
202	0.192	0.00904	12.8	47
203	0.261	0.02131	19.0	82
204	0.110	0.00557	15.4	51
205	0.292	0.01757	35.0	60
206	0.270	0.02741	27.6	102
mean	0.238	0.01683	14.8	67

Table XVI. Gastric secretion of frogs which are fastened in a clamp. Secretion elicited by distension of the stomach (r.s.s.= 2; duration: 2 hours). Slow intravenous injection of Ringer's solution (28 ml/kg.) and KOMAROV's gastrin (dose corresponding to 20 g. of fresh tissue per kg.). Mean bodyweight: 44 g.

No	volume (ml.)		total HCl (m.eq.)		pepsin (P.U.)		acidity (mN)	
	Ringer	gastrin	Ringer	gastrin	Ringer	gastrin	Ringer	gastrin
209	0.353	0.353	0.01678	0.00941	18.6	10.1	48	27
210	0.518	0.315	0.01835	0.02142	14.4	26.9	35	68
211	0.353	0.276	0.00431	0.00969	4.8	6.9	12	35
212	0.364	0.277	0.00908	0.01078	8.4	10.7	25	39
213	0.374	0.360	0.00430	0.01281	3.5	8.2	11	36
214	0.347	0.181	0.00960	0.00660	4.9	5.6	28	36
mean	0.385	0.294	0.01040	0.01179	9.1	11.4	27	40

Table XVII. Secretory output of frogs with destroyed c.n.s. Secretion evoked by mechanical stimulation (r.s.s.= 2; duration: 2 hours) after injection of KOMAROV's gastrin (corresponding to 20 g. of fresh mucosal tissue/kg.) or histamine (0.5 mg. histamine-diHCl/kg.).

volume (ml.)	total HCl (m.eq.)	pepsin (P.U.)	acidity (mN)	
0.207	0.00156	7.8	8	
0.117	0.00147	4.6	13	
0.225	0.00234	13.0	10	gastrin subcut.
0.176	0.00153	2.2	9	
0.150	0.00154	9.0	10	
0.175	0.00169	7.4	10	
0.144	0.00121	2.4	9	
0.166	0.00173	6.8	10	
0.139	0.00115	4.4	8	gastrin i.v.
0.142	0.00117	4.0	8	
0.264	0.00317	7.6	12	
0.171	0.00169	5.0	9	
0.136	0.00606	4.0	45	
0.146	0.00076	6.8	5	
0.287	0.00324	3.2	11	histamine i.v.
0.243	0.00776	8.2	32	
0.123	0.00179	2.6	15	
0.187	0.00392	5.0	22	

Table XVIII. Secretory output of intact frogs in which secretion has been evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration: 2 hours). Mean bodyweight: 55.4 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
174	0.435	0.00512	0.01221	0.00700	19.8	12	28	16	46
175	0.232	0.00764	0.01066	0.00302	6.2	33	46	13	27
188	0.708	0.00459	0.01192	0.00733	21.5	6	17	10	30
199	0.346	0	0.00494	0.00494	17.6	0	14	14	51
202	0.301	0	0.00360	0.00360	12.3	0	12	12	41
206	0.787	0.01307	0.02143	0.00836	11.9	17	27	11	15
207	0.447	0.00819	0.01228	0.00409	21.9	18	27	9	49
174	0.349	0.01018	0.01477	0.00459	13.0	29	42	13	37
175	0.345	0.00866	0.01297	0.00431	6.6	25	38	12	19
188	0.620	0.00927	0.01748	0.00821	57.1	15	28	13	92
199	0.324	0.01861	0.02473	0.00612	19.7	57	76	19	61
202	0.223	0.01250	0.01717	0.00467	25.9	56	77	21	116
206	0.338	0.02107	0.02618	0.00511	7.7	62	77	15	23
207	0.478	0.01897	0.02740	0.00843	22.7	40	57	18	47
mean	0.424	0.00985	0.01555	0.00570	18.9	26	40	14	47

Table XIX. Gastric secretory output during distension of the stomach following subcutaneous injection of GREGORY's gastrin stage I (r.s.s.= 2; duration: 2 hours; gastrin dose corresponding to 75 g. of fresh tissue per kg.). Mean bodyweight: 55.4 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
174	0.178	0.01421	0.01934	0.00513	7.7	80	109	29	43
175	0.291	0.01152	0.01550	0.00398	7.1	40	53	14	24
188	0.503	0.01493	0.02229	0.00736	24.5	30	44	15	49
199	0.321	0.00852	0.01380	0.00528	8.2	27	43	16	26
202	0.433	0.01775	0.02497	0.00722	8.0	41	58	17	18
206	0.386	0.02601	0.03196	0.00595	9.7	67	83	15	25
207	0.507	0.01405	0.02230	0.00825	26.9	28	44	16	53
174	0.464	0.01125	0.01812	0.00687	15.3	24	39	15	33
175	0.569	0.02137	0.02854	0.00717	9.0	38	50	13	16
188	0.585	0.00181	0.00861	0.00680	28.6	3	15	12	49
199	0.239	0.00841	0.01122	0.00281	16.2	35	47	12	68
202	0.361	0.02067	0.02870	0.00803	19.6	57	80	22	54
206	0.270	0.01608	0.02071	0.00463	12.0	60	77	17	44
207	0.693	0.02502	0.03437	0.00935	16.8	36	50	13	24
mean	0.414	0.01511	0.02146	0.00635	15.0	40	57	16	38

Table XX. Secretory output of intact frogs in which secretion has been evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration: 2 hours). Mean bodyweight: 55.5 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
173	0.298	0.02381	0.03250	0.00869	13.9	80	109	29	47
174	0.678	0.00000	0.01461	0.01461	23.3	0	22	22	34
175	0.234	0.01321	0.01828	0.00507	9.0	56	78	22	38
186	0.295	0.00634	0.01143	0.00509	15.9	21	39	17	54
188	0.356	0.01428	0.02114	0.00686	25.6	40	59	19	72
193	0.431	0.01938	0.02800	0.00862	16.0	45	65	20	37
197	0.242	0.01829	0.02418	0.00589	2.3	76	100	24	10
199	0.363	0.01113	0.01831	0.00718	9.7	31	50	20	27
201	0.412	0.01972	0.02786	0.00814	10.0	48	68	20	24
202	0.215	0.01021	0.01457	0.00436	4.0	47	68	20	19
203	0.281	0.01306	0.01891	0.00585	14.5	46	67	21	52
204	0.443	0.01629	0.02273	0.00644	25.6	37	51	15	58
205	0.296	0.00916	0.01419	0.00503	9.8	31	48	17	33
206	0.336	0.01602	0.02082	0.00480	5.1	48	62	14	15
207	0.378	0.01099	0.01874	0.00775	8.5	29	50	21	22
mean	0.351	0.01346	0.02042	0.00696	12.9	42	62	20	36

Table XXI. Gastric secretory output during distension of the stomach following subcutaneous injection of GREGORY's gastrin stage III (r.s.s.= 2; duration: 2 hours). Mean bodyweight: 55.5 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
173	0.364	0.03701	0.04567	0.00866	6.8	102	125	24	19
174	0.590	0.02202	0.03928	0.01726	14.6	37	67	29	25
175	0.449	0.01837	0.02516	0.00679	6.3	41	56	15	14
186	0.390	0.01922	0.02580	0.00658	8.0	49	66	17	21
188	0.491	0.02828	0.03281	0.00453	30.5	58	67	9	62
193	0.514	0.01717	0.03077	0.01360	24.2	33	60	26	47
197	0.399	0.02522	0.03100	0.00578	13.0	63	78	14	33
199	0.311	0.02115	0.02596	0.00481	10.2	68	83	15	33
201	0.454	0.02664	0.03689	0.01025	23.3	59	81	23	51
202	0.378	0.02833	0.03338	0.00505	7.7	75	88	13	20
203	0.340	0.02491	0.02967	0.00476	9.7	73	87	14	29
204	0.433	0.01305	0.01984	0.00679	11.9	30	46	16	27
205	0.478	0.01506	0.02117	0.00611	16.7	32	44	13	35
206	0.337	0.02228	0.02714	0.00486	13.7	66	81	14	41
207	0.330	0.01886	0.02484	0.00598	14.8	57	75	18	45
mean	0.417	0.02250	0.02996	0.00745	14.1	56	74	17	33

Table XXII. Pepsin output (P.U.) after application of a small mechanical stimulus to the stomach (r.s.s.= 1) and a larger mechanical stimulus (r.s.s. = 3). Duration: 4 hours.

bodyweight in grams	Pepsin output (P.U.) r.s.s. = 1	r.s.s. = 3
25	4.0; 3.6; 4.2	45.8; 35.7; 12.8
31	15.4; 18.2; 62.8	2.0; 13.1
33	5.5; 2.3	
37	7.9; 0.4; 0.2	46.6
40	1.2; 0.6; 1.2	
43	7.1; 21.2	6.0; 48.0; 50.5
50	6.7; 1.2; 10.5	
55	3.4	41.0
62	4.2	8.4; 30.6; 46.4
68	1.0; 1.6	37.6
70	6.6; 4.7	8.8; 13.0; 80.0
80	4.2; 63.2	5.1; 53.0
92	0.4; 4.7	
50	9.3	30.8

Table XXIII. Secretory output of intact frogs in response to distension of the stomach (r.s.s.= 2; duration: 1.5 hours.) Mean bodyweight: 53 grams.

Number	volume (ml.)	free HCl (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	pepsin conc. (PU/ml.)
172	0.098	0.01393	21.4	142	218
173	0.234	0.02025	9.8	87	42
174	0.136	0.00878	21.4	65	158
175	0.198	0.01031	13.8	52	70
176	0.092	0.00665	10.8	71	118
186	0.108	0.01113	27.8	103	258
188	0.526	0.01427	15.2	27	28
189	0.179	0.00843	13.8	47	78
191	0.146	0.00980	24.2	67	166
192	0.127	0.01857	21.0	146	166
172	0.125	0.01873	24.0	150	192
173	0.332	0.02185	15.4	66	46
174	0.133	0.00636	22.8	48	172
175	0.125	0.00968	9.2	77	74
176	0.383	0.00000	10.2	0	26
186	0.180	0.00589	30.0	32	166
189	0.284	0.00657	19.6	23	68
191	0.332	0.00702	25.6	21	78
mean	0.208	0.01101	18.6	68	118

Table XXIV. Acid and pepsin secretion of intact frogs following subcutaneous injection of atropine. Secretion evoked by mechanical stimulation of the stomach. (r.s.s.= 2; duration: 1.5 hours; 20 mg. of atropine sulphate per kg.). Mean bodyweight: 48 grams.

Number	volume (ml.)	free HCl (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	pepsin conc. (PU/ml.)
173	0.290	0.01055	5.8	36	20
174	0.236	0.00745	10.8	32	46
175	0.227	0.00392	5.4	17	24
176	0.340	0.00142	6.4	4	18
186	0.184	0.00624	17.6	34	96
188	0.349	0.01240	17.6	36	50
189	0.223	0.00597	13.6	27	60
191	0.240	0	15.8	0	66
192	0.237	0.00941	10.0	40	42
mean	0.258	0.00637	11.4	25	46

Table XXV. Secretory output in frogs in which secretion has been evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration: 2 hours). Mean bodyweight: 45.2 g.

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
174	0.267	0	0.00344	0.00530	6.8	0	13	20	25
175	0.190	0.01724	0.02352	0.00628	7.6	91	124	33	40
186	0.171	0.01198	0.01957	0.00759	34.0	70	114	44	198
195	0.251	0	0.00465	0.00465	7.3	0	19	19	29
197	0.318	0.01617	0.02349	0.00732	19.2	51	74	23	60
200	0.435	0.01379	0.02210	0.00831	18.9	32	51	19	43
130	0.587	0.01287	0.01823	0.00536	15.6	22	31	9	27
213	0.227	0.01334	0.02010	0.00676	16.3	59	89	30	72
214	0.383	0.00448	0.01140	0.00692	14.3	12	30	18	37
215	0.396	0.02061	0.02803	0.00742	16.9	52	71	19	43
216	0.273	0.00574	0.00973	0.00399	--	21	36	15	--
217	0.563	0.00286	0.01053	0.00767	14.2	5	19	14	25
mean	0.339	0.00992	0.01624	0.00647	15.6	35	56	21	54

Table XXVI. Gastric secretion of frogs which are fastened in a clamp. Secretion evoked by distension of the stomach (r.s.s.= 2; duration: 2 hours). Mean bodyweight: 45.2 g. (Data are to be compared with those of Table XXV.)

Number	volume (ml.)	free HCl (m.eq.)	total HCl (m.eq.)	buffer (m.eq.)	pepsin (P.U.)	free HCl conc. (mN)	total HCl conc. (mN)	buffer conc. (mN)	pepsin conc. (PU/ml.)
174	0.321	0	0.00194	0.01274	8.4	0	6	40	26
175	0.133	0	0.00262	0.00347	4.2	0	19	26	32
186	0.143	0	0.00303	0.00529	10.5	0	21	30	73
195	0.173	0	0.00096	0.00589	7.2	0	6	34	42
197	0.111	0	0.00173	0.00314	2.9	0	16	28	26
200	0.397	0.00790	0.01613	0.00823	23.3	20	41	21	59
130	0.207	0	0.00190	0.00882	5.6	0	9	43	27
213	0.194	0	0.00294	0.00510	11.7	0	15	26	60
214	0.406	0.01394	0.02123	0.00729	16.1	34	52	18	40
215	0.133	0	0.00162	0.00390	4.4	0	12	29	33
216	0.131	0	0.00150	0.00414	3.8	0	11	32	29
217	0.230	0	0.00157	0.00739	3.3	0	7	32	14
mean	0.215	0.00182	0.00477	0.00629	8.5	5	18	30	39

Table XXVII. Pepsin output (P.U.) of frogs with damaged c.n.s. Secretion evoked by mechanical stimulation of the stomach (r.s.s.= 2; duration: 4 hours). 35 expts. Mean bodyweight: 45 grams.

Section through medulla oblongata	brain destroyed	c.n.s. pithed
18.1	14.2	1.0
10.9	2.1	1.0
29.8	14.6	6.7
10.9	3.6	0.5
16.0	2.4	4.6
7.2	16.3	8.1
10.9	3.2	0.0
4.3	4.2	5.1
3.6	7.0	0.0
	5.8	4.8
	6.0	1.4
	10.4	
	22.4	
	1.6	
	18.6	
Mean 12.4	8.8	3.0

Table XXVIII. Pepsin output (P.U.) of control frogs and of Nembutal anaesthetized animals. Mean bodyweight: 45 grams.

Control. r.s.s.= 2 4 hours	Control. Stomach drained.	Nembutal. r.s.s.= 2 2 hours
29.8	4.8	10.2
49.9	1.4	1.9
40.2	0.7	19.3
88.0	1.1	0.8
94.0	2.2	2.5
70.9	0.8	4.3
46.5	0.9	16.2
82.6	0.8	7.6
26.9	0.6	1.1
23.5	0.0	1.2
92.6	0.9	4.1
82.1	0.8	1.9
34.9	0.7	4.8
52.9	0.6	4.1
21.7	0.7	2.5
50.8	0.7	3.8
34.9	0.6	0.8
89.2	0.7	4.9
21.7	0.7	9.1
87.0	0.6	
	0.5	
56.0	1.0	5.3

Table XXIX. Pepsin yield (P.U.) after drainage of the stomach, which previously has been ligated at the pyloric end, in controls and animals with denervated stomachs. 27 expts.

Controls	Stomach denervated
14.9	0.7
2.0	1.0
2.9	0.7
1.3	1.0
0.0	4.6
2.5	1.3
0.0	0.8
1.9	0.5
1.7	1.0
2.8	4.4
2.3	6.4
2.6	1.5
2.5	1.7
3.3	
2.9	2.0

Table XXX. Pepsin output (P.U.) of jumping frogs with and without mechanical stimulation of the stomach. Mean bodyweight: 45 grams. 41 expts.

r.s.s.= 2 2 hours	stomach drained after 2 hours jumping
5.4	2.0
12.0	3.2
16.2	3.3
10.4	2.9
27.2	1.1
9.6	1.3
8.4	1.9
29.6	1.7
5.3	4.2
10.9	1.8
12.9	3.2
15.0	1.7
22.8	1.5
16.0	2.8
18.8	2.0
11.7	1.9
25.7	0.9
36.0	3.1
21.4	2.5
3.4	2.2
30.7	
16.6	2.3

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